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8637 C*

Cyanide Inhibition of Frog Tissues.

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Some time ago it was reported that cyanide produces practically complete inhibition of nerve respiration and that when lower grades of inhibition were observed this was attributable in large part to escape of cyanide into the alkali in the inset.¹ Recently it became desirable to construct a vessel which would permit a study of cyanide inhibition in which preliminary determinations of resting respiration could be carried out in the complete absence of cyanide from the vessel. This was accomplished by the use of a dosing stopcock to contain the cyanide which was placed in the bottom of the vessel so that at any moment by turning this stopcock cyanide would diffuse into the fluid bathing the tissue. When

* C represents a complete, P a preliminary manuscript.

¹ Schmitt, F. O., and Schmitt, O. H. A., *Am. J. Physiol.*, 1931, **97**, 302.

cyanide inhibition of frog nerve respiration was studied, not only was the previous report of complete inhibition confirmed, but even very low concentrations of cyanide had considerable effect. Such low concentrations could not be studied with the previous method because of escape of HCN. The inhibitions obtained with other tissues were also higher than those reported by others.

At the outset of the experiment the hollow dosing stopcock, greased and inserted into the female member with the opening facing downwards, is completely filled with neutralized buffered sodium cyanide by means of a capillary pipette. The stopcock is then turned through 90°. The nerves or other tissue to be studied are then placed in the main vessel in 1.0 cc. of phosphate Ringer, 5% KOH being placed in the side arm for absorption of CO₂. The vessels are placed on differential manometers and a curve of resting respiration determined. After obtaining a satisfactory line for resting respiration the dosing stopcocks of the control and experimental vessels are turned so that the opening is now to the vessels permitting diffusion of cyanide into the fluid around the tissue. The usual result is an immediate decrease of respiration which, after 15 to 20 minutes, with concentrations of cyanide greater than about 0.0004 M becomes practically complete inhibition (96-100%). With low concentrations of cyanide the inhibition begins to wear off after an hour or 2 and eventually disappears entirely. There is seldom any tendency for tissues such as nerves to fall through the hole into the dosing stopcock. If desirable a small irregularly shaped glass pearl may be placed over the hole to prevent this in the case of more finely divided tissue.

TABLE I.
Cyanide Inhibition of Respiration of Frog Tissue. T = 19-22° C.

Tissue	Maximum Equilibrium Concentration of NaCN M	Maximum Inhibition %
Nerve	.001	98
"	.0005	99
"	.0002	81
"	.00009	46
"	.00001	27
Kidney	.01	94
"	.005	89
"	.001	79
"	.0005	57
Stomach	.01	94
"	.005	85
Intestine	.001	78

Aside from the fact that with this vessel the resting respiration of the tissue may be first determined in the complete absence of cyanide, there is the further advantage that any escape of cyanide into the alkali of the inset must take place by diffusion through the medium bathing the tissue. The latter point appears to be of considerable importance for, as shown in Table I, with this method the inhibitions obtained with tissues such as kidney, stomach and intestine are considerably higher than those usually reported for these tissues. For example Dixon and Elliott² and Muntwyler and Binns³ obtained a maximum inhibition of 60-80% for these tissues whereas with this method, inhibitions of 85-95% have been obtained regularly with 0.01 to 0.05 M NaCN. In view of the convenience of this method and the considerably higher degree of inhibition obtained with it as compared with the conventional method it has been adopted in this laboratory for all experiments on the effect of cyanide or other volatile reagents upon tissue respiration.

8638 P

Action of Drugs, Nerves and Electric Current in Iris Sphincter.

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It was thought that electrical stimulation might help to locate the site of action of a neural hormone.

A ring or strip a few millimeters wide was cut from the pupillary margin of the iris, usually of a cat, and hung to a writing lever between platinum electrodes in a modified Locke solution of pH 7.4, warmed to about 37°C. The sphincter thus isolated was stimulated electrically, usually for about 5 seconds at a time, with a faradic current from a Harvard inductorium using 3 fresh dry cells in the primary.

The accompanying sample tracing illustrates the findings that:

Electrical stimulation, 1E, of the isolated sphincter muscle caused a contraction followed by a much greater relaxation. This suggests that the electric current "stimulated" first a contractor re-

² Dixon, M., and Elliott, K. A. C., *Biochem. J.*, 1929, **23**, 812.

³ Muntwyler, E., and Binns, D., *Am. J. Physiol.*, 1934, **108**, 80.

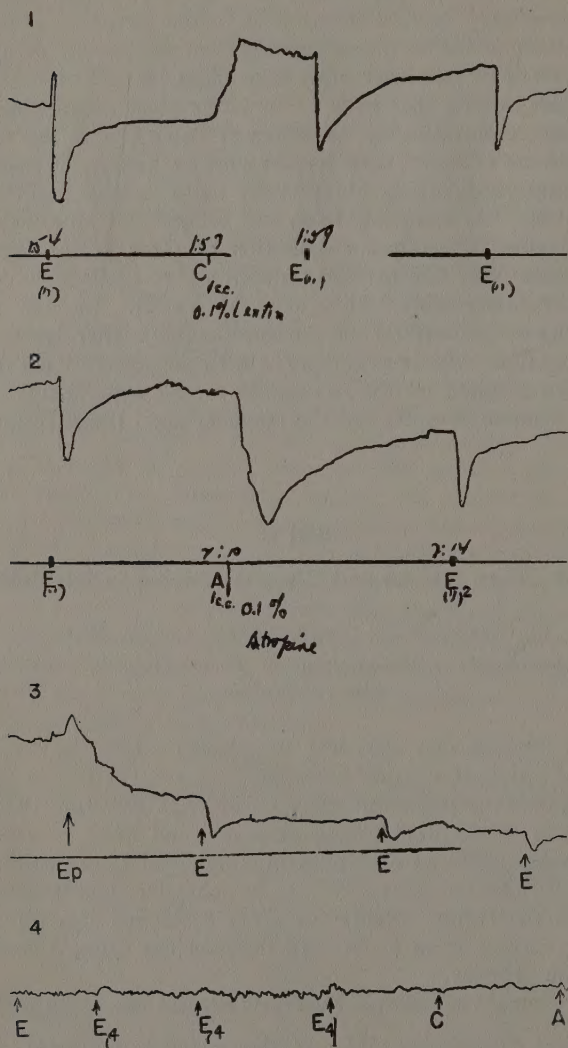


FIG. 1.

Photostat of tracing, retouched. Series 2, Exp. 20, Cat No. 14, 1/29/36. E, Elect. Stim., coil Dist. 6, for 5 sec.; C, 1 cc. of 0.1% Carbam. Chol.; A, 1 cc. Atrop., 0.1%; E_p , 1 cc. Epineph., 1:10,000, Segment 4 follows two more doses of Epineph. Here the base line is level with the tracing. E_4 , Elect. Stim. w. C. D. 4 cm. The minute waves represent spontaneous contractions or tremor of the apparatus.

ceptive substance and then a relaxor receptive substance in the sphincter muscle.

Carbaminoyl choline, 1C, now produced a sustained contraction, and electrical stimulation still produced a contraction with much greater relaxation. Since this choline ester, in this concentration, does not act on nerve endings (Shaklee and Christensen¹), the action must be on the contractile mechanism or on some other substance in the muscle fiber.

Atropine, 2A, after the choline ester, caused the disappearance of both contractions, *i. e.*, that produced by the choline ester and that produced by electrical stimulation. The inference seems to be that atropine inhibited the contractor receptive substance, upon which both the choline ester and the electric current seem to act. Electric current, 2E₂, after the atropine, caused only relaxation and about as much as before the drugs had been applied. This would seem to indicate that neither the choline ester nor the atropine, present in the bath, was affecting the contractile mechanism directly, or the relaxor receptive substance; hence they must have acted on the contractor receptive substance.

Epinephrine, 3Ep, added to the choline ester and atropine, already in the bath, caused a further relaxation, presumably by "stimulating" the relaxor receptive substance upon which the electric current had been acting. *Excess* epinephrine caused a disappearance of the relaxation due to electric current, 4E. From this, one might infer that excess epinephrine exhausted or inhibited the relaxor receptor substance upon which it and the electric current had been acting.

Increased electric current, 4E₄, after both the contractor and the relaxor receptive substances had been inhibited by atropine and epinephrine, caused a slight contraction followed by an equal relaxation. Since the receptive substances had been nullified and since the contraction was followed by an equal relaxation, this contraction seems to be due to direct stimulation of the contractile mechanism by the electric current.

The increase in length of the muscle after atropine and epinephrine suggests that most of the normal tone of isolated smooth muscle is due to spontaneous catalysis of the receptor substance.

In this study we have used eyes from 46 cats, 2 rabbits and 2 pigs. Further details will follow.*

¹ Shaklee, A. O., and Christensen, K., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1619.

* We are grateful to Merck and Co., for the carbaminoyl choline.

Summary: The results of electric stimulations of an isolated smooth muscle before and after the successive applications of a choline ester, atropine and epinephrine, seem to lend support to the theory that these drugs and the electric current, in ordinary strengths, act solely on receptive substances for the neural hormones. These findings are in harmony with the chemical theory of nerve transmission, and, taken with the findings of others, suggest that each axone in the body acts by liberating at its endings a specific substance that catalyzes a specific receptive substance in the effector or other nerve cell with which they lie in contact, into fractions, one of which directly stimulates or inhibits the effector mechanism, or, in the nerve cell, the impulse mechanism. The experiments of Láncoz² suggest calcium ions as one of these fractions, those of Brown and Feldberg³ suggest potassium ions as another.

8639 C

Influence of Estrone upon Galactin Content of Male Rat Pituitaries.*

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McQueen-Williams¹ has demonstrated that one normal male rat pituitary contains sufficient galactin to induce a good response in a one-month-old pigeon when implanted subcutaneously over the crop gland. Better results have been obtained in our laboratory by injecting the macerated pituitary intradermally over the crop gland as described by Lyons and Page.²

In order to study the effect of estrone upon the galactin content of male rat pituitaries, 16 animals were paired upon the basis of body weight. One of each pair was injected subcutaneously daily for 15 days with 500 International Units of the benzoate of hy-

² Láncoz, Anna, *A. f. Exp. Path. u. Pharm.*, 1936, **180**, 312.

³ Brown, G. W., and Feldberg, W., *J. Physiol.*, 1936, **86**, 10P.

* Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series No. 464.

¹ McQueen-Williams, M., *Proc. Soc. Exp. Biol. and Med.*, 1935, **33**, 406.

² Lyons, W. R., and Page, Emery, *Proc. Soc. Exp. Biol. and Med.*, 1935, **32**, 1049.

TABLE I.

Rat No.	Body Wt., gm.	Total Pituitary Wt., mg.	Pigeon Body Wt., gm.	Sex	Assay Data	
					Crop Gland Rating	
					Right	Left
9*	160	6.2	303	M	0.5	0
16†	156	4.8	284	M	1.5	0
3*	161	4.7	272	F	1.0	0
5†	162	5.6	299	M	1.0	0
10*	164	5.0	310	F	0.5	0
15†	167	6.9	295	M	1.5	0
7*	168	4.8	273	F	0.75	0
18†	172	6.5	296	M	1.0	0
8*	180	6.0	292	F	0.75	0
4†	182	6.0	289	F	1.0	0
6*	206	5.5	292	M	0.75	0
14†	199	6.8	331	M	1.0	0
13*	214	5.5	270	M	0.75	0
12†	216	7.1	289	M	1.5	0
11*	241	7.6	281	F	0.75	0
2†	222	7.2	339	F	1.25	0

*Control animals.

†Experimental animals.

doxyestrin (Oestroform B). At the end of 15 days all of the animals were sacrificed, their pituitaries removed and weighed. Each pituitary was then macerated, using an agate mortar and pestle, and suspended in 0.4 cc. of distilled water. This suspension was then injected intradermally over the right crop gland of a common rock pigeon at the rate of 0.1 cc. per day. The left crop gland was used as a control for each rat pituitary. The pigeons were sacrificed at 96 hours, weighed, and their crop glands removed. The crop glands were rated by both of us according to the degree of proliferation with one of us not knowing their identity. The results are summarized in Table I.

Of these 8 pairs of rats, 7 of the pituitaries from the animals under the influence of Oestroform B contained more galactin than their control mates and the eighth experimental animal's pituitary was rated as equal to that of its control mate.

These data indicate that the normal male rat pituitary contains an appreciable amount of the lactogenic hormone and that the injection of the estrogenic hormone definitely increases the galactin content of the pituitary. These observations do not indicate whether the increase is due to more rapid secretion of galactin or to the prevention of its discharge into the blood stream.

Effect of Hypophysectomy and Replacement Therapy on Lactation in Guinea Pigs.*

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Under favorable conditions, lactation has been induced experimentally in the normal rabbit, dog, sow, guinea pig, cat, cow and goat by means of the lactogenic hormone. Hypophysectomy in late pregnancy or during lactation either prevents the initiation¹ or causes the rapid cessation of lactation.² In hypophysectomized dogs,^{3, 4} cats and ferrets,⁵ the induction of lactation has been reported to be stimulated by extracts of the anterior pituitary gland containing the lactogenic hormone.

The present study was aimed to determine the immediate effect of hypophysectomy on lactation and of replacement therapy in the initiation or maintenance of lactation in the guinea pig. Hypophysectomized lactating females and experimentally cryptorchid males carrying functional ovarian grafts from 6 to 8 weeks were used. The operation, carried on by the parapharyngeal route, was well tolerated and the high post-operative mortality experienced in earlier work was successfully controlled by the daily administration of glucose.

Seven lactating guinea pigs were hypophysectomized during the first week after delivery. Lactation at this time was well established and would normally continue for 2 to 3 weeks. All the animals showed a rapid decline in milk secretion the first day after hypophysectomy, being completely dry in 2 to 3 days. These observations confirm and extend the report of Nelson¹ in which the cessation of lactation followed hypophysectomy in 2 animals. Five

* Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series No. 465.

† Aided in part by a grant of the Committee on Grants-in-Aid of the National Research Council.

¹ Nelson, W. O., *Proc. Soc. Exp. Biol. and Med.*, 1935, **33**, 222.

² Selye, H., Collip, J. B., and Thompson, D. L., *Proc. Soc. Exp. Biol. and Med.*, 1933, **30**, 589; **31**, 82.

³ Evans, E. D., *U. S. D. A. Yearbook*, 1934, 360.

⁴ Houssay, B. A., *Comp. rend. Soc. Biol.*, 1935, **120**, 496.

⁵ McPhail, M. K., *Proc. Roy. Soc. B.*, 1935, **117**, 34, 45.

of these animals were given the purified lactogenic hormone† (galactin) in amounts varying from 20 to 50 mg. daily for periods from 9 to 12 days immediately after the cessation of milk secretion. The 5 injected and the 2 uninjected animals showed no evidence of secretion during a period of 10 days. The glands at autopsy and later verified by microscopic examination showed marked involution of the glandular parenchyma.

In addition, 6 animals which upon autopsy were found to be incompletely hypophysectomized, showed a decline in milk secretion during the first few days following hypophysectomy, but soon returned to a level which was sufficient to raise their young without other feed. The glands of these animals at autopsy showed abundant milk secretion.

A total of 10 lactating females was hypophysectomized. Four were given galactin by subcutaneous injection in varying amounts from 10 to 40 mg. immediately after hypophysectomy and continued daily thereafter for 10 days. Three of the animals were completely dry in 2 to 4 days after hypophysectomy, while the fourth was in milk throughout the observation period. At autopsy, the latter animal was found to be incompletely hypophysectomized. Two were given 10 mg. of acetone dried anterior pituitary powder daily immediately after hypophysectomy. Both animals died on the third and fourth days, respectively, after the first injection. The glands at the time of death were well hypertrophied but secretion was present only in the lumina of the large ducts. Two were given a ground aqueous suspension of $\frac{1}{4}$ of a fresh whole sheep pituitary gland daily. One of the animals died 10 days after the first injection and the second was sacrificed after 20 days. The glands of both animals at autopsy were engorged with milk comparable to normal lactating females. One animal was given 0.5 cc. of an alkaline extract (1 cc. = 1.8 gm. fresh tissue) daily. Lactation declined the day after operation. On the fourth day milk was no longer present in the glands.

Five unilaterally castrated and experimentally cryptorchid male guinea pigs carrying functional ovarian grafts for 6 to 8 weeks were hypophysectomized. (In these animals the existence of functional ovarian grafts in the cryptorchid testis causes a complete development of the mammary gland. In intact animals, the injection of galactin or the removal of the testis-grafts is followed by the secretion of milk.⁶) Three of the animals were given 10 mg.

† Three daily injections of 5 mg. of this preparation produced copious lactation in experimentally cryptorchid males carrying functional ovarian grafts.

⁶ Turner, C. W., and Gomez, E. T., *Mo. Agr. Exp. Sta. Res. Bul.*, 206, 1934.

of galactin daily beginning immediately after hypophysectomy, while in the other 2 the testis was removed. In addition, 3 cryptorchid intact males were given 5 mg. daily as controls. One of the injected hypophysectomized animals died on the second day after the first injection and the other two 6 days later. The untreated animals were sacrificed after 10 days. Lactation did not occur in any of the hypophysectomized animals. Lactation was well established after 3 daily injections in the intact cryptorchid males.

Summary. Hypophysectomy in lactating guinea pigs is immediately followed by a rapid decline in lactation and a complete cessation within 2 to 3 days after operation. Galactin was not able to initiate lactation in hypophysectomized experimentally cryptorchid males or to re-initiate and maintain lactation following hypophysectomy at any stage during the first week of lactation. The injection of a suspension of ground fresh whole sheep pituitary gland tissue was able to maintain lactation in 2 cases.

The failure of galactin to initiate or maintain lactation in the hypophysectomized guinea pig is believed to emphasize the importance of the products of the other endocrine glands controlled by the hypophysis in lactation. The importance of the thyroid⁷ and of the adrenal cortex⁸ has been indicated. Carbohydrate metabolism is also intimately concerned in milk secretion.

8641 P

Maintenance of Carbohydrate Levels in Fasted Hypophysectomized Rats Treated with Anterior Pituitary Extracts.*

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Herbert M. Evans.)

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It has previously been reported by one of us¹ that hypophysectomized rats, while they have normal carbohydrate levels in

⁷ Graham, W. R., Jr., *Biochem. J.*, 1934, **28**, 1368.

⁸ Gaunt, R., and Tobin, C. E., *Anat. Rec. (Supp.)*, 1936, **64**, 18.

* Aided by the Presnell Fund for the study of the metabolic relations of the anterior hypophysis contributed by Robert R. Presnell, Frank Tuttle, Arthur Stebbins and Mrs. Gordon Kahn, of Los Angeles.

¹ Russell, J. A., *PROC. SOC. EXP. BIOL. AND MED.*, 1936, **34**, 279.

TABLE I.
Carbohydrate Levels* in Fasted Hypophysectomized Rats Treated with Anterior Pituitary Extracts.

	No. of Animals	Blood Glucose mg. %		Liver Glycogen mg. %		Muscle Glycogen mg. %	
		Aver.	Range	Aver.	Range	Aver.	Range
I. Fasted 8 hours							
1. Unoperated controls	6	100 $\pm$ 3	89-113	1210 $\pm$ 210	420-2620	490 $\pm$ 16	440-566
2. Untreated hypophysectomized control	7	60 $\pm$ 2	48-71	17 $\pm$ 1	15-18	445 $\pm$ 15	369-558
3. W6027 (alk. extract) .5 cc. in 24 hrs.	7	99 $\pm$ 4	74-119	541 $\pm$ 130	71-1470	617 $\pm$ 8	594-700
4. Treatment† over 3 days	8	87 $\pm$ 5	44-147	620 $\pm$ 150	15-1940	611 $\pm$ 16	502-700
II. Fasted 24 hrs.							
1. Unoperated controls	10	71 $\pm$ 2	62-83	26 $\pm$ 3		529 $\pm$ 4	513-550
2. Untreated hypophysectomized controls	7	42 $\pm$ 2	30-50	13 $\pm$ 1		354 $\pm$ 10	302-418
3. W6027 .5 cc. in 24 hrs.	8	53 $\pm$ 3	36-75	13 $\pm$ 2		578 $\pm$ 15	496-651
4. W6027 .1 cc. per day, 4-6 days.	4	54	45-73	15		566	542-592
III. Adrenalectomized-hypophysectomized. Fasted 24 hrs.							
W6027 .5-.7 cc. in 48-72 hrs.	6	35 $\pm$ 2	28-45	17 $\pm$ 3	5-26	545 $\pm$ 21	427-653

*Details of determinations as reported previously.

†In most cases 0.7 = 1.0 cc. of W6027 total dosage

blood, liver, and muscle when fed, do not maintain these levels during fasting as well as do normal animals. With the administration of anterior pituitary extracts, we have been able to prevent this abnormal fall in carbohydrate levels in short fasting periods.

The results of the most important series in these experiments have been summarized in Table I.

The extract (W 6027) used in most cases in this work was prepared routinely in the laboratory.† It contained 17 mg. of organic matter per cubic centimeter, each cubic centimeter being equivalent to about 130 mg. of fresh tissue.

It was found necessary to give the extracts in divided doses, spread over 18 to 24 hours for the best effects. When the same amount of total material was given in one dose 8 or 24 hours before the determinations of carbohydrate levels were made, no effect was obtained. Subcutaneous injections, however, were found to be no more effective than the intraperitoneal route usually used. Shorter periods of treatment than 18 hours were less effective, and more chronic treatment (3 to 6 days) was not found more effective than the 24-hour period.

In addition to the extract W6027, which contains all the known anterior lobe hormones, 2 fractions high in growth hormone§ and one high in adrenotropic effect|| but also containing fairly large amounts of growth hormone were used on 8 hypophysectomized rats and found to be very active in preserving glycogen stores. The 2 growth preparations were free of gonadotropic and mammatropic effects as shown by immature pigeon tests, and one of these preparations as well as the adrenotropic fraction appeared to contain no thyrotropic hormone. These hormones would thus appear to be ruled out in this action of the anterior pituitary. Although this "glycostatic" factor appears to accompany the growth hormone, its relation to this hormone is not established.

In 6 cases the right adrenal was removed a few days prior to hypophysectomy and the left adrenal 11 to 19 days after hypophysectomy. Treatment with the standard alkaline extract, W6027, was instituted immediately after the removal of the second adrenal

† A given amount of frozen, ground beef hypophyses is taken up in 7½ volumes of 0.04 N Ba(OH)₂, extracted in the ice box 18 hours and then passed 4 times through a brine-cooled Sharples centrifuge. The supernatant is neutralized to pH 8.0 with 0.2 N H₂SO₄ and any excess barium precipitated out by the addition of a solution of Na₂SO₄. The solution is again centrifuged and the supernatant is ready for use.

§ Prepared in this laboratory by Doctors N. Uyei and Q. Bartz.

|| Prepared in this laboratory by Mr. Henry Moon.

and the animal sacrificed in 48 or 72 hours after a 24-hour fast. The glycogen stores in these animals do not differ significantly from those in the other treated 24-hour fasted, hypophysectomized rats. It would thus seem that this effect cannot be exerted through the adrenal cortex.

Two preparations, W6027 and the adrenotropic fraction, when heated to boiling for one hour, were without activity of any sort in 4 rats.

The method by which these anterior pituitary preparations act to preserve carbohydrate stores is unknown. New formation of carbohydrate was not demonstrated when, in a few animals, attempts were made to raise fasting levels already low; nor when the extract was given to unfasted animals were abnormally high levels found. A possible gluconeogenetic action of the anterior lobe is not ruled out, however, by the experiments.

It may be significant that the muscle glycogen content seems to be primarily affected in this action of the anterior lobe and that the effects on blood and liver do not necessarily follow those on the muscle. Although there is a general correspondence between the 3 values, in many individual cases marked hypoglycemia and very low liver glycogen levels have been observed accompanied by muscle glycogen figures between 650 and 700 mg. % in 8-hour and 550-620 mg. % in 24-hour fasted treated rats, values well above those encountered in normal controls.

Summary: The abnormal fall in body carbohydrate suffered by hypophysectomized rats during fasting can be prevented by the administration of anterior pituitary extracts, and the muscle glycogen values can be raised to supernormal levels for the fasted conditions. This action of the anterior pituitary occurs in the absence of the adrenal glands.

Reactivation of Thermally Inactivated Bacteriophage.

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It has been shown by Anson and Mirsky¹ in a long series of studies that the denaturation of many proteins is a reversible process. The isolation of crystalline trypsin² and pepsin³ has furnished proof that at least some enzymes are protein compounds. In them the denaturative reaction is paralleled by loss of enzymatic activity and conversely, as the denatured protein is changed back to native protein this activity is regained.⁴

Krueger⁵ found that the thermal inactivation of phage follows the course of a unimolecular reaction and that the critical thermal increment for inactivation is about 100,000. Since critical thermal increments of this order of magnitude are uniquely characteristic of protein-denaturation he concluded that the inactivation of phage by heat involved protein-denaturation as a significant reaction. Additional experimental support for this concept is available in other observations:

1. Vedder⁶ has noted that the loss of activity on heating dried phage is much less than with phage in aqueous suspension; dried proteins are typically more thermostable than proteins in solution.

2. While immunological experiments are not theoretically conclusive they are most readily interpreted by considering phage to be a protein capable of functioning as an antigen.⁷

3. Purified phage gives the usual chemical tests for proteins and on elementary analysis has been found to contain carbon, hydrogen, nitrogen and phosphorus^{8,9} in proportions empirically compatible with a protein structure.

Taken together these data suggest that phage is composed of

¹ Anson, M. L., and Mirsky, A., Publications in *J. Gen. Physiol.*, beginning with 1925, **9**, 169.

² Northrop, J. H., and Kunitz, M., *J. Gen. Physiol.*, 1932, **16**, 267.

³ Northrop, J. H., *J. Gen. Physiol.*, 1930, **13**, 739.

⁴ Northrop, J. H., *J. Gen. Physiol.*, 1932, **16**, 323.

⁵ Krueger, A. P., *J. Gen. Physiol.*, 1932, **15**, 363.

⁶ Vedder, A., *Centralbl. f. Bakt.*, I, Orig., 1932, **125**, 111.

⁷ Krueger, A. P., *Physiol. Rev.*, 1936, **16**, 129.

⁸ Schlesinger, M., *Biochem. Z.*, 1934, **273**, 254.

⁹ Meyer, Karl, Thompson, R., Khorazo, D., and Palmer, J. W., *Proc. Soc. Exp. Biol. and Med.*, 1935, **33**, 129.

one or more proteins and that its lytic activity is intimately linked with maintenance of the native protein structure. This would be entirely in keeping with the view of some workers that phage is an enzyme and it must be admitted that certain analogies exist between the properties of phage and those of enzymes. For example, phage can be quantitatively reactivated after complete inactivation by concentrated HgCl_2 ,¹⁰ or by HCN ¹¹ if the toxic Hg or CN ions are removed by chemical means. The action of phage on its bacterial substrate is accelerated by the presence of certain cations in very minute concentrations.¹² Such phenomena, among others, are common both to phage and to enzymes. However, as one of us has pointed out⁷ they fail to afford formal proof of identity simply because the characteristics of living cells in general are largely determined by the enzymes they elaborate and as a consequence many analogous properties are shared by cells and their enzymes. It has not been possible, therefore, definitely to disprove the theory that phage is a special sort of living ultramicrobe and to relegate it to the class of enzymes by reasoning based on the analogies between the reactions of phage and enzymes.

Since it is known that the heat-inactivation of phage is closely connected with protein-denaturation it should be possible to reverse the reaction. If then phagic activity is dependent upon the native state of protein present there should be an accompanying regeneration of this activity. In the present paper we wish to present experimental evidence indicating that the thermal inactivation of phage actually is reversible.

To 24 ml. of standard phage, containing 1×10^{10} activity units,¹³ in beef-infusion broth at pH 7.4 was added 5.4 ml. of HCl-phthalate buffer of pH 3.2. The mixture had a final pH of 5.75 and was heated at 54°C. for 20 minutes. At the end of this time a sample (A) was removed and cooled as rapidly as possible in a salt-ice mixture. Another 9.8 ml. sample (B) was added to 9.0 ml. of chilled broth containing enough NaOH to bring the mixture to a final pH of 8.1. Both samples were kept on ice for 16 hours and then tested for phage by the activity-titration method.¹³ As controls, aliquots of unheated phage were adjusted to pH 5.75 (C) and to pH 8.1 (D) as noted above. A third control (E) consisted of unheated phage adjusted to pH 5.75 for 20 minutes and then brought to

¹⁰ Krueger, A. P., and Baldwin, D. M., *J. Infect. Dis.*, 1935, **57**, 207.

¹¹ Krueger, A. P., and Elberg, S. S., *PROC. SOC. EXP. BIOL. AND MED.*, 1934, **31**, 483.

¹² Krueger, A. P., and West, N. S., *J. Gen. Physiol.*, 1935, **19**, 75.

¹³ Krueger, A. P., *J. Gen. Physiol.*, 1930, **13**, 557.

pH 8.1. All controls were prepared at 10°C. and were kept at ice-box temperature for 16 hours before titration.

In all, 24 successful experiments were performed with the following average results (after correcting for dilution):

Sample A contained 20% of the original phage present.

Sample B contained between 70% and 85% of the original phage.

Controls C, D and E showed no effect from exposure to pH 5.75 and pH 8.1 at 10°C. for 16 hours.

From the above data it is evident that alkalization and rapid cooling combine to increase the activity of phage suspensions partially inactivated by heat. In view of the fact that the thermal inactivation of phage has been shown to involve protein-denaturation our results are probably best interpreted by assuming that the denaturation and concomitant inactivation occurring when phage is heated at pH 5.75 are in part reversed at pH 8.1. It may be objected that the apparent increase in activity is due to the effect of an increased $[\text{OH}^-]$ on residual active phage remaining after the heating and has nothing to do with the inactivated fraction. Our controls prove that this is not the case for in E no change in [phage] could be demonstrated. Similarly, the possibility that the regeneration of activity may involve a stimulating effect on the titrated mixtures by ions introduced with the buffers, must be considered. If this were the case the time of lysis in the titration sample would be less and the solution would have an apparently increased titre. Again, the controls, C, D and E indicate that no such effect is obtained with either buffer singly or when mixed as is done in reversing the denaturation.

The most logical conclusion is that the thermal inactivation of phage is quite analogous to that of enzymes. In the case of pure trypsin in solution the enzyme can be heated almost to boiling without significant loss of activity as long as the pH is maintained well on the acid side.² Northrop³ has shown that this remarkable thermoresistance is due to the influence of the H ion in favoring the reversal of denaturation. Krueger and Scribner¹⁴ have described the catalyzing effect of H and OH ions on the thermal inactivation of phage. Their data together with those reported here, indicate that when phage is inactivated by heat (denaturation) the pH of the solution during the period of heat treatment and during cooling has a most important bearing on the final titre obtained. Under some circumstances a considerable proportion of the phage so in-

¹⁴ Krueger, A. P., and Scribner, E. J., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **33**, 21.

activated may again be activated. This the authors interpret as contributing further to the analogies already existing between phage and enzymes. While this still does not prove that phage is an enzyme, it should be emphasized that there no longer exist valid philosophical objections to entertaining such a consideration. Stanley's¹⁵ isolation of a crystalline protein possessing all the properties of tobacco-mosaic virus is proof that there exist inanimate substances which though lethal for certain cells may yet possess the capacity for being propagated by contact with these cells. It is not altogether illogical to speak of phage in such terms.

8643 P

Comparative Effects of Pancreas and Choline on Blood Cholesterol of Depancreatized Dog Maintained with Insulin.*

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It was shown¹ that the completely depancreatized dog can survive for well over 4 years when maintained with insulin and a diet containing meat, sucrose, bone ash and vitamin supplements, B (in the form of a rice bran concentrate), and A and D (as cod liver oil). No other accessory substances were found essential. Despite their survival, however, such dogs are not normal in all respects. The appearance of cataracts was first observed in this laboratory as early as one year after pancreatectomy.² A striking change also takes place in the lipid metabolism. When the animals are maintained on the dietary constituents listed above, there occurs a fall in all blood lipid constituents and a rise in the lipid content of the liver.^{3, 4} It was then shown that the addition of raw pancreas to the diet not only decreased the lipid content of the

¹⁵ Stanley, W. M., *Science*, 1935, **81**, 644.

* Aided by a grant from the Research Board of the University of California, Berkeley. The insulin used in this investigation was generously supplied by Eli Lilly and Company.

¹ Chaikoff, I. L., *Proc. Soc. Exp. Biol. and Med.*, 1935, **33**, 211.

² Chaikoff, I. L., and Lachman, G. S., *Proc. Soc. Exp. Biol. and Med.*, 1933, **31**, 237.

³ Chaikoff, I. L., and Kaplan, A., *J. Biol. Chem.*, 1934, **106**, 267.

⁴ Kaplan, A., and Chaikoff, I. L., *J. Biol. Chem.*, 1935, **108**, 201.

liver to the normal level but also effected a rise in the blood lipids to values far in excess of the preoperative or normal level.⁵ The maintenance of a high blood lipid level was observed only so long as raw pancreas was being ingested, the exclusion of the glandular tissue from the diet resulting in a precipitous fall in the lipid level, which finally reached a value below the normal. The ability of raw pancreas, when administered *per os*, to cause these fluctuations in the blood lipids, as well as its effect on the lipid metabolism of the liver, suggested that *this glandular tissue contained a substance—entirely apart from insulin—capable of influencing lipid metabolism.* Since choline has been claimed to effect a cure of the fatty livers

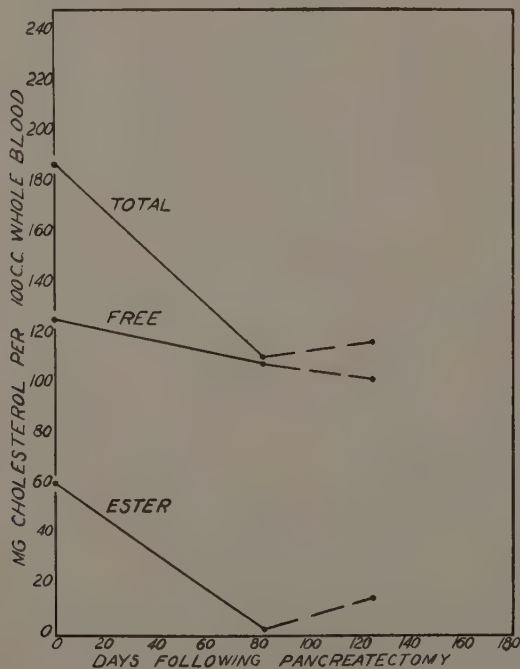


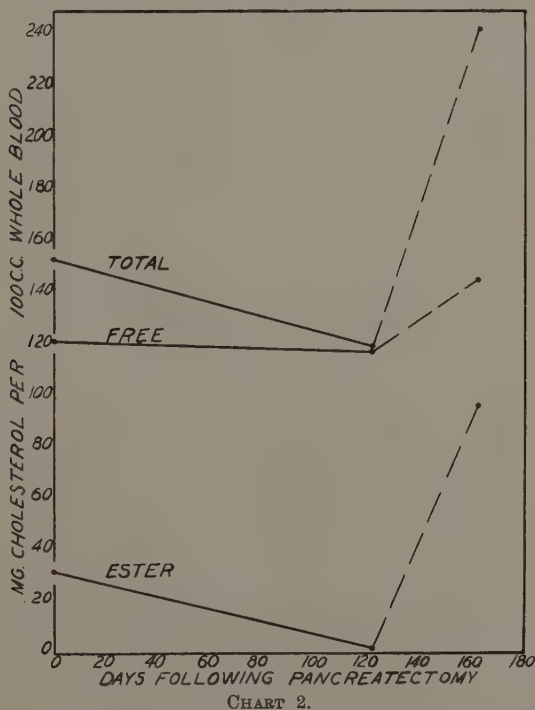
CHART 1.

Effect of choline on blood cholesterol of Dog D23. Solid line represents blood cholesterol during period in which the dog ingested meat, sucrose, bone ash and vitamin supplements. Broken line shows cholesterol level during period in which 2 gm. of choline hydrochloride was ingested daily along with the above dietary constituents.

⁵ Chaikoff, I. L., and Kaplan, A., *J. Biol. Chem.*, 1935, **112**, 155.

of the depancreatized dog,⁶ it became necessary, in the process of identifying the pancreatic lipid factor, to study the effects of choline on the blood lipids of the completely depancreatized dog maintained with insulin.

The dietary treatment of the depancreatized dogs maintained in this laboratory has been described elsewhere.^{3,5} Blood studies were made on 5 dogs receiving choline in their diets and on 10 dogs receiving raw pancreas. A typical result of each is recorded. Dog D23 was depancreatized on September 26, 1934, and D24 on September 27, 1934. Both animals were fed a diet containing none of the glandular tissue for a period sufficient to produce a marked lowering of the blood lipids. In the case of D23, a marked de-



Effect of raw pancreas on blood cholesterol of Dog D24. Solid line represents blood cholesterol during period in which the dog ingested meat, sucrose, bone ash and vitamin supplements. Broken line shows level of blood cholesterol during period in which 250 gm. of raw pancreas was ingested daily along with the above dietary constituents.

⁶ Best, C. H., Ferguson, G. C., and Hershey, J. M., *J. Physiol.*, 1933, **79**, 94.

crease in the cholesterol content of the blood was observed on December 17, 1934, and for a period of 6 weeks after this date 2 gm. of choline hydrochloride were added daily to the diet. In D24 a lowered cholesterol content of the blood was established on January 27, 1935, and for a period of 6 weeks thereafter the diet of this animal was supplemented with 250 gm. of raw pancreas daily.

The results obtained are shown in Charts 1 and 2. The addition of raw pancreas to the diet of D24 produced a rise in the cholesterol esters from 2 to 97 mg. and in the total cholesterol a rise from 120 to 243 mg.% in the space of 40 days. The administration of choline to D23 produced very little change in the cholesterol level of the blood. It has been emphasized by us previously³ that a notable effect of the pancreatic lipid factor is its ability to raise and maintain a cholesterol level in the blood, both total and esterified, far above the normal. The addition of choline to the diet of D23, in amounts far greater than the choline contained in the lecithin content of raw pancreas fed D24, failed to raise the total and esterified cholesterol content of the blood of D23 from a lowered level to a level above the preoperative or normal.

8644 C

Pancreatic Diabetes and Hypophysectomy in the Rat.

R. SHAPIRO AND GREGORY PINCUS. (Introduced by Frederick L. Hisaw.)

From the Biological Laboratories, Harvard University

The laboratory mammals used for pancreatectomy are the dog and the cat. The complete extirpation of the pancreas of the rat and related rodents is considered an impossibility because of the diffuse nature of this organ and the inaccessibility of certain branches of tissue. Nonetheless it seemed a matter of some interest and usefulness to investigate the effects of as complete an extirpation as is possible.

The rat pancreas consists roughly of 3 portions: (1) the head, or duodenal division located on the right of a line following the portal vein from the junction of the splenic and superior mesenteric veins to the point at which it passes behind the first part of the duodenum; (2) the tail, or splenic, division located on the left of a line passing from the junction of the splenic and superior mesen-

teric veins along the left border of the omental border of the pancreas; and (3) the body, or mid-portion, consisting of the rest of the pancreas between these 2 lines. We find that we can remove completely the tail and body and most of the head with the exception of certain tissues located in the mesenteries of part of the duodenum and transverse colon. This can be accomplished by an electro-cautery which is used to sever the exposed parts close to the duodenum and especially for cauterizing one by one the small blood vessels passing from the spleen into the tail of the pancreas. Ordinary methods of blood vessel ligation are unusable. The animals used were about 30 days old at operation since at this age in the rat the maximum islet number is attained (Overholser¹).

This operation should result in the removal of the great majority of the pancreatic islets since Overholser¹ has shown that in a linear unit of the head the islet number is 18.3, in the body 19.0, and in the tail 34.0. In cubic units the tail alone therefore contains $3\frac{1}{2}$ times the number of any other portion.

Blood sugar measurements were made on all the animals used before the operation and on a considerable number of control, unoperated animals. Sufficient blood for a microdetermination (method of Folin²) was obtained by heart puncture. Heart punctures can, with practise, be completed in 30 seconds without exciting the animals. The normal blood sugar concentration of the rats raised upon our standard laboratory diet is 101.8 mg. % and the

TABLE I.*
The Blood Sugar Concentrations of Rats at Various Intervals After Pancreatectomy and When Followed by Hypophysectomy.

Days after Pancreatectomy	No. of Animals	Mean blood Sugar	Standard Deviation
0	73	101.81	7.083
4	10	135.92	12.303
5	21	172.26	9.981
6	14	183.56	11.952
7 $\frac{1}{2}$	23	199.88	15.604
13	15	216.57	17.866
18 $\frac{1}{2}$	22	213.18	11.366
25 $\frac{1}{2}$	18	211.42	10.643
34	25	218.79	11.704
62	16	211.81	12.600
3	3	180.72	—
5	9	169.10	—
12 $\frac{1}{2}$	7	112.79	—
24	7	98.39	—

*Days after hypophysectomy are indicated below the blank line.

¹ Overholser, M. O., *Endocr.*, 1925, **9**, 493.

² Folin, O., *N. E. J. Med.*, 1932, **206**, 727.

low coefficient of variation (7%, see Table I) of the determinations taken by heart puncture indicates the reliability of this method.

After pancreatectomy the blood sugars of the operated animals were taken at intervals over a period of 2 months. These data are summarized in Table I and presented graphically in Fig. 1A. It

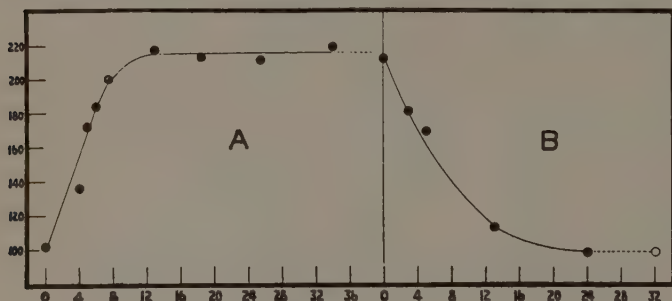


FIG. 1.

Ordinates: blood sugar in mg. %. 1A abscissa: time in days after pancreatectomy. 1B abscissa: time in days after hypophysectomy of pancreatectomized rats. The 0-day value for curve 1B is the mean blood sugar concentration at 62 days after pancreatectomy; the 32-day value is the mean of a group of 20 rats which had attained blood sugar equilibrium.

will be seen that there is a prompt rise in blood sugar concentration to a maximum of about 215 mg. % by 2 weeks after pancreatectomy, and that thereafter this level is constantly maintained. The constancy of this level is indicated by the fact that the coefficient of variation is about 5%, the extreme values, after the second week, being 190 and 233 mg. % respectively. These data imply therefore that there is no regeneration of islet tissue and that the remaining unexcised islets are sufficient to keep the blood sugar at a constant determined level. It should be noted that aside from a small mortality at operation due to occasional hemorrhage or overanæsthetization the animals recovered and remained perfectly healthy thereafter gaining weight and showing no detectable superficial symptoms. There were no deaths due to uncontrollable hyperglycemia and no indications of the attainment of lethal levels.

Hypophysectomy of the depancreatized animals results in a restoration of normal blood sugar levels (Table I and Fig. 1B). It is notable that the return to the equilibrium level (about 98 mg. %) also occurs in about 2 weeks. We find that among the animals hypophysectomized after the maximum hyperglycemic level has been attained there was a greater mortality at operation than in a group hypophysectomized at 4 to 6 days after pancreatectomy.

The equilibrium blood sugar level of the hypophysectomized-pancreatectomized animals is slightly more variable than that of normal animals. In a group of 20 depancreatized animals the blood sugars of which were taken at 24 to 42 days after hypophysectomy the mean level was 97.87 with a coefficient of variation of about 9% and extreme values of 84 and 117 mg. % respectively.

In attempting hypophysectomies to be followed by pancreatectomies we noted that a number of the hypophysectomized rats died within a few days and the rest within a few weeks. These deaths proved to be due to hypoglycemia, for in a group of hypophysectomized animals observed at 8 days after hypophysectomy and raised upon our standard diet the mean blood sugar was 73.02 mg. % whereas in a control group operated on at the same time but given additional sugar in the diet the mean value was 102.18 mg. %.

Summary: A constant hyperglycemic blood sugar level of about 215 mg. % is attained in about 2 weeks after removal of the tail, body, and most of the head portion of the pancreas of month-old rats. Hypophysectomy restores the blood sugar level to about 98 mg. % which is in the normal range (102 mg. %). Hypophysectomy without pancreatectomy results in hypoglycemia unless sugar additional to that used in the diet of normal rats is added.

8645 C

Bacteriologic Studies in Myasthenia Gravis.

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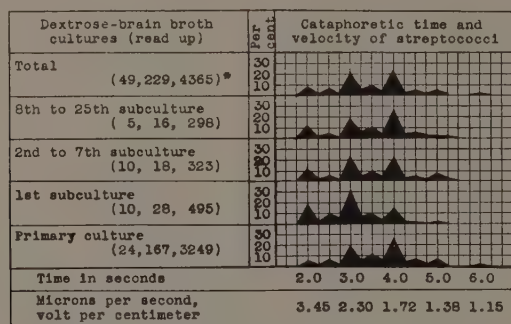
Boothby and his associates¹ have reported on their metabolic studies in cases of myasthenia gravis and on the beneficial effect of treatment with glycine and ephedrine. They have called attention to the fact that not infrequently the disease begins, or is made worse, following upper respiratory infections. We have coöperated in the study of this peculiar syndrome and have carried out bacteriologic studies in 49 cases by methods similar to those previously described.^{3, 4} A streptococcus which was very similar in

¹ Boothby, W. M., *J. Am. Med. Assn.*, 1934, **102**, 259.

³ Rosenow, E. C., *Internat. Clin.*, 1930, **2**, 29.

⁴ Rosenow, E. C., and Jensen, L. B., *J. Infect. Dis.*, 1933, **52**, 167.

the different cases has been consistently isolated from the nasopharynx and other foci of infection, and sometimes from the muscles and urine of patients with myasthenia gravis. This streptococcus, although resembling in its morphologic and cultural characteristics green-producing streptococci isolated from similar sources in cases of diseases other than those affecting muscles and nerves, differed markedly from these green-producing streptococci in its cataphoretic velocity (Fig. 1) and in its specific virulence.



*The figures in parenthesis indicate respectively, the number of strains, cultures, and streptococci timed in each group studied.

FIG. 1.
Cataphoretic time and velocity of the streptococcus of myasthenia gravis.

The primary blood-agar plate-cultures of material from the nasopharynx and other foci of infection were not characteristic, but the number of colonies of green-producing streptococci was usually far greater than in like cultures from well persons. This was true before, as well as after, weakness of muscles of the pharynx became evident. The distribution of cataphoretic velocities of the streptococci, as isolated in dextrose-brain broth, was of the "neuro-myotropic" type (Fig. 1) in practically all cases and varied but little according to season.

We have isolated the streptococcus in pure culture from excised pieces of muscle in 3 acute cases of myasthenia gravis during the patients' lives, and from 3 pieces of muscle excised 1½ hours after the patient's death in a fourth case. We have also demonstrated diplococci² within or adjacent to lesions in the muscles in each of 5 severe cases of myasthenia gravis (Fig. 2). Cultures and sections of muscles in a series of controls proved negative.

² Butt, H. R., *Arch. Path.*, 1936, **21**, 27.

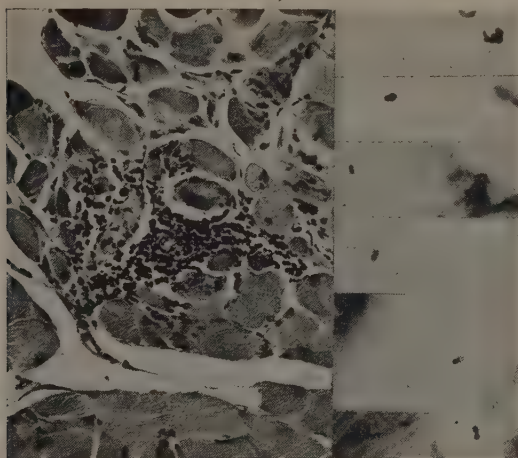


FIG. 2.

Section of muscle from a patient who had myasthenia gravis. A circumscribed area of lymphocytic collections (lymphorrhagia) is evident (hematoxylin and eosin stain, $\times 175$) and diplococci can be seen in, or adjacent to, the lesions (Gram-Rosenow stain, $\times 1000$).

The most striking characteristics of the streptococcus on isolation in dextrose-brain broth were its marked tendency, following intravenous and intracerebral injection, to localize and to produce lesions in muscles, often involving nerve fibrils, to grow slowly, and to survive for a long time in muscular tissue, causing soon or long after injection progressive weakness, undue fatigue on exertion, and characteristic microscopic lesions in the muscles. The streptococcus was isolated from and demonstrated in the lesions of muscles in monkeys (Fig. 3) and rabbits (Fig. 4) when the blood and other tissues were free from streptococci as long as 4 months after intravenous injection, but this was true only in animals that showed symptoms resembling myasthenia gravis. The heat-killed streptococcus, filtrates of cultures, and solutions of the partially-purified specific carbohydrate of the streptococcus, just as the living culture, electively produced characteristic lesions in muscles following intravenous and intracerebral injection; the weakness and undue fatigue on exertion, however, were transient. The resistance of animals decreased following repeated intravenous and intracerebral injection of the living streptococcus but increased following repeated injections of the heat-killed streptococcus and of filtrates of cultures and filtrates of glycerin-salt solution extracts of the streptococcus. Patients with myasthenia gravis were found to be ex-

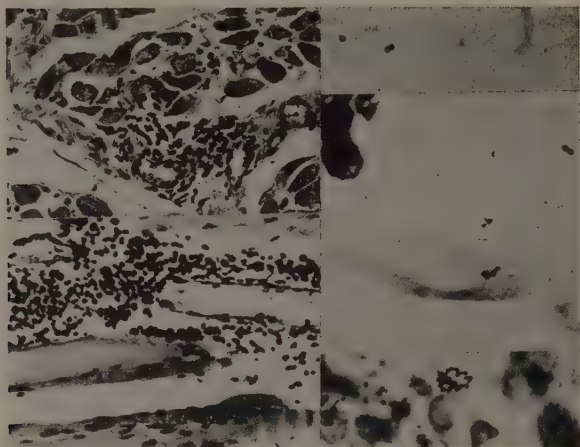


FIG. 3.

Cross and longitudinal sections of muscles of the monkey referred to in the protocol. Areas of lymphocytic collections (lymphorrhagia) are evident (hematoxylin and eosin stain, $\times 175$), and diplococci can be seen in lesions in skeletal muscles and myocardium (Gram-Rosenow stain, $\times 1000$).



FIG. 4.

Granular, hyaloid, and fatty degeneration and lymphocytic collections in and between muscle fibers (a, b, hematoxylin and eosin stain, $\times 175$), and diplococci in the lesions in muscles of the rabbit referred to in the protocol (c, gram stain, $\times 1000$).

tremely sensitive to therapeutic subcutaneous injections of the heat-killed streptococcus and filtrates. The manifestations following injection of the heat-killed streptococcus and of filtrates of cultures occurred promptly and were attributed to a specific toxin or poison of the streptococcus and not to "virus."

The living streptococcus on isolation produced symptoms of myasthenia gravis and characteristic lesions in the muscles of monkeys and rabbits, after many (25) rapidly repeated subcultures and after from one to 4 passages through animals. Affinity of the streptococcus for muscles was greatest in the strains isolated from excised pieces of muscle, and this affinity increased on passage of such strains through rabbits if the streptococcus was isolated consecutively from the muscles of positive animals. Specific localizing power and other distinctive properties of the streptococcus usually disappeared promptly on cultivation in ordinary mediums.

Control animals injected intravenously and intracerebrally with streptococci from diseases not involving muscles never developed symptoms or lesions in muscles such as those following like injection of the streptococcus of myasthenia gravis.

The results obtained following intravenous injection of the living streptococcus of myasthenia gravis into monkeys and rabbits are well illustrated in the following protocols:

1. A *Macacus rhesus* monkey was given intravenous injections on 3 occasions, 3 days apart, of 40 cc. of the dextrose-brain-broth culture of a strain, after one passage through a rabbit and 14 subcultures in dextrose-brain broth, from the muscle of a patient. Aside from moderate weakness, from which the animal recovered soon after these injections, it remained apparently well for 3 months. Rapidly progressing weakness and undue fatigue on exertion then developed. Four months after injection, when the animal was anesthetized, it was entirely unable to stand or to chew or swallow its food, even banana, on account of profound weakness. Diarrhea and generalized edema of the subcutaneous tissues had developed. The muscles of the jaw had become so weak that the lower jaw could easily be pressed open with one's fingers. Consciousness was normal and the animal tried repeatedly to move about when prodded. Aside from marked atrophy of the muscles and subcutaneous edema no lesions were found at necropsy. Cultures from the brain and blood remained sterile. Dextrose-brain-broth cultures of pieces of muscle excised under sterile precautions yielded green-producing streptococci which had the characteristic cataphoretic velocities and which produced progressive weakness

and characteristic lesions in the muscles of rabbits following intravenous injection. Sections of muscles revealed typical mild, diffuse, and dense, sharply-circumscribed areas of lymphocytic accumulations (lymphorrhagia) and few diplococci (Fig. 3).

2. A white rabbit was given an intravenous injection August 30, 1934, of 12 cc. of the primary dextrose-brain-broth culture of the streptococcus from the tonsil of a patient who had myasthenia gravis. The animal seemed well 3 days later, September 2, when the injection was repeated. On September 3 it appeared well but, on slight exercise, the front legs spread outward owing to weakness and fatigue. September 4 it was weaker and more easily exhausted. On September 5 it again appeared well but after only moderate exertion it became completely exhausted. September 7 it appeared well when sitting in its cage but again became easily exhausted on exertion. It was given 2 cc. intravenously, and 2 cc. subcutaneously, of the same strain as before, but after this strain had been passed through 2 rabbits. On September 11 the animal was still easily exhausted. Eight cubic centimeters of the dextrose-brain-broth culture were injected intravenously. On September 19 the animal was weaker, became very easily fatigued, and lay with its legs outstretched after slight exertion. September 22 it was very weak; slight exercise exhausted it completely and it lay on its back unconcerned when placed there. By October 17, the animal had become extremely weak and almost powerless in its hind extremities. On November 1 it was almost powerless in its hind extremities but could move them. On November 3 there was almost complete absence of strength in the hind extremities and there was marked weakness of the fore extremities and of the muscles of the neck; it could not hold its head erect. It attempted repeatedly to get up on its legs but was entirely unable to do so on account of marked weakness. It was therefore etherized. On examination there was marked generalized atrophy of muscles, in some of which soft, edematous, whitish, friable areas were found. Cultures from the blood, brain, fluid from knee-joints, and urine remained sterile; those from the muscles revealed a pure culture of the streptococcus. Sections of muscles revealed marked hyaloid, fatty, and granular degeneration of muscular fibers associated with edema and lymphocytic collections and few diplococci (Fig. 4).

Summary. By the methods which have been successfully used in a study of the etiology of other diseases a streptococcus of low general virulence, having characteristic cataphoretic velocity, has been found constantly associated with cases of myasthenia gravis.

The living and heat-killed streptococcus and its toxins have specific affinity for muscles. The progressive weakness, fatigability, and the pathologic lesions of myasthenia gravis have been reproduced in monkeys and rabbits.

8646 C

Sulfur Partitions in Cat Urines Following Injections of Monobromobenzene, Cystine or Methionine.*

ROBERT W. VIRTUE. (Introduced by H. H. Beard.)

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Monohalobenzenes have been found to be conjugated with cysteine and excreted as mercapturic acids in the urine of the dog, rabbit, rat¹ and mouse.² The work herein reported was undertaken to see whether the cat could also effect such a synthesis. It also became of interest to carry out distributions of urinary sulfur after administration of cystine or methionine.

Cats were kept in metabolism cages, and urines collected at 24-hour intervals by gentle abdominal pressure. In the analysis, sulfur determinations were run by Folin's method, nitrogen by Kjeldahl, and disulfide compounds according to Virtue and Lewis.³ The cats were put on their dietary regime for 7 days before collection of urine was begun, and urines were analyzed 4 days before injections were made. To insure that a constant supply of the material whose metabolism was to be studied would be present in the body, all injections were subcutaneous, and were given as follows: half the amount 24 hours before the end of the metabolism period, one-fourth 6 hours before the end, and the remaining fourth 3 hours later. The metabolism period was limited to 24 hours because the animals were sacrificed at the end of that time to obtain information for other work. Thirty-three animals were used in the series. Typical results are shown in Table I.

As will be seen in the figures for cats 29 and 37, the organic

*Part of these data were presented before the American Society of Biological Chemistry at Washington, D. C., March 25-28, 1936.

¹ Lawrie, N. R., *Biochem. J.*, 1931, **25**, 1037.

² Stekol, J. A., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **33**, 115.

³ Virtue, R. W., and Lewis, H. B., *J. Biol. Chem.*, 1934, **104**, 415.

TABLE I.
Distribution of Urinary Sulfur of Cats Following Subcutaneous Injection of Monobromobenzene, Methionine and Cystine.

Cat.	Dietary Regime	Total N mg.	Total S mg.	Sulfate S mg.	Inorganic Sulfate-S mg.	Organic S mg.	Ethereal Sulfate-S mg.	Org. S	
								Total S	%
29	Fasting	1058	56	39	32	17	7		
		1039	56	39	36	17	3		
		1004	56	39	34	17	5		30
		953	57	40	34	17	6		
		*1655	85	48	20	37	28		
37	Meat Scraps	2339	123	59	40	64	23	} §	
		2339	123	59	40	64	23		
		2594	141	70	49	71	21		51
		2202	111	55	39	56	16		
		*2486	140	60	21	80	39		
9	Fasting	958	64	48	39	16	9		
		985	64	48	39	16	9		26
		963	55	41	35	14	6		
		864	49	35	30	14	5		
		†1005	120	92	88	28	4		
	"Extra S"		62	49	52	13	—3		
21	Fasting	1192	80	38		42			
		1118	71	35		36			
		1273	78	42		36			49
		1329	91	52		39			
		†1561	242	159		83			
	"Extra S"		162	117		45			

*1.5 gm. bromobenzene (in olive oil) per kilo; half at 24 hours, a quarter each at 6 and 3 hours before end of 24-hour period.

†Methionine, equivalent to 250 mg. S, given as sodium salt; half 24 hours, a quarter each at 6 and 3 hours before end of 24-hour period.

‡Cystine, equivalent to 250 mg. S, given as sodium salt; half 24 hours, a quarter each at 6 and 3 hours before end of 24-hour period.

§Analyzed together.

sulfur rose when either fasted or well-fed cats were given monobromobenzene. It seems justifiable to assume that this rise, at least in part, was due to the formation of mercapturic acids, for on treatment of these urines according to the method of McGuinn and Sherwin,⁴ white crystalline substances melting at 153° (Corr.) were obtained. When mixed with p-bromophenylmercapturic acid which had been isolated from rat urine† no depression of the melting points resulted.

⁴ McGuinn, A., and Sherwin, C. P., *Proc. Soc. Exp. Biol. and Med.*, 1933, **30**, 1115.

† The author wishes to express his thanks to Dr. Abraham White for a gift of the p-Bromophenylmercapturic acid from rat urine.

Since the nitrogen output was greatly increased after administration of monobromobenzene to fasting animals (Cat 29), it appears likely that the cats broke down their own tissues to obtain the cysteine necessary for the synthesis of mercapturic acids. High nitrogen values following injections were not obtained consistently with well-fed cats. (Cat 37).

The values reported for urines from cats 9 and 21 indicate that the "extra" sulfur from both cystine and methionine was for the most part excreted in the form of sulfate sulfur by this species. However, part of the "extra" organic sulfur from cystine (Cat 21) must have been excreted unchanged, for the urine of this animal gave a positive Sullivan reaction.

It has been suggested⁵ that demethylation may be a step in the metabolism of methionine, with the subsequent formation of homocysteine, and by oxidation, homocystine. A test for disulfide groups other than cystine has been reported in the urine of animals which had been given methionine.⁵ Analyses of urine for disulfide compounds were made, therefore, before and after injections of methionine. Calculated as homocystine, daily pre-experimental averages on 2 of these animals were 5 and 8 mg. respectively, while after injection of methionine these values rose to 16 and 22 mg. These urines gave negative Sullivan reactions for cystine.

The high proportion of organic sulfur excreted by the cat is noteworthy. The values for the pre-injection periods of the 4 animals included in Table I were 30, 51, 26 and 49%, respectively, and the average values for the entire series was 33% or higher. This is a much larger proportion of organic sulfur than has been found in normal urines from the dog or rabbit.

No other sulfur partitions on cat urines have come to the attention of the author.

Conclusions: 1. Fasting and well-fed cats excreted p-bromophenylmercapturic acid in their urines following the injection of monobromobenzene. 2. Cysteine necessary for the synthesis of mercapturic acids by fasting cats may arise from their own tissues. 3. Sulfur partitions have been made on urines from cats.

⁵ Virtue, R. W., and Lewis, H. B., *J. Biol. Chem.*, 1934, **104**, 59.

A Physiological Solution for Freshwater Crustaceans.

A. VAN HARREVELD. (Introduced by T. H. Morgan.)

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Contrary to what we know of the nerve muscle preparation of the frog, the irritability of the nerve of a freshwater crustacean quickly disappears on bathing it in an isotonic sodium chloride solution. For marine crustaceans seawater may be used successfully as a physiological solution. For *Astacus fluviatilis*, Hoffmann's¹ formula, a solution of about twice the concentration of the Ringer solution for the frog, has generally been employed. With such solutions the results with nerve muscle preparations have not been very satisfactory (Hoffmann, Segaar²). With the crustacean heart, however, the results have been better (Potioné,³ Lindemann⁴). An attempt was made to find a better physiological solution for the freshwater crayfish.

Two species of freshwater crustaceans, *Astacus trowbridgii* and *Cambarus clarkii* were investigated. The freezing point of the blood was determined in order to know the molecular concentration. The amount of the physiologically important ions, Ca, K and Mg was estimated, and finally the pH was determined. As even large specimens do not give more than .5 to 1 cc. of blood, the blood of 8 to 10 crayfishes was collected and mixed, and all the estimations except the pH were done with this. The figures obtained in this way are thus averages, for our purpose not a disadvantage. The blood was collected by sucking it out of the pericardial cavity which surrounds the heart ventricle, with a paraffined hypodermic syringe. The blood for the Ca, K and Mg determination and for the freezing point was cooled to 0° and stirred for about 15 minutes to prevent clotting. After centrifuging a clear serum was obtained. The blood for the pH determination was collected in a small amount of a 1% potassium oxalate solution under a layer of liquid paraffin, to avoid loss of CO.²

The freezing point determination of the serum was made with an ordinary Beckmann thermometer. For the estimation of the

¹Hoffmann, P., *Z. f. Biol.*, 1914, **63**, 411.

²Segaar, J., *Z. f. vergl. Physiol.*, 1929, **10**, 120.

³Potioné, H., *Z. f. vergl. Physiol.*, 1926, **3**, 528.

⁴Lindemann, V. F., *Physiol. Zool.*, 1928, **1**, 576; 1929, **2**, 395.

Ca, K and Mg ions the procedure of Kramer and Tisdall⁵ was used. To determine the Ca, the modification by Clark and Collip⁶ was chosen. The K determination was done exactly as described by Kramer and Tisdall. For the Mg determination their method was slightly modified. A precipitate of ammonium-magnesium-phosphate was formed and washed in the way described by Tschopp,⁷ and the phosphate in the precipitate was estimated with the colorimetric method of Kuttner and Cohen.⁸ Determining the Ca, K and Mg in a solution of known concentration, the methods for Ca and K were found to be accurate within 5%, that for Mg within 10%. The pH determination was done with a glass electrode.

In Tables I and II figures obtained from the blood of *Astacus trowbridgii* and of *Cambarus clarkii* are given. For both species 3 different groups of animals obtained at different times were examined. The blood of each group was taken more than once, with an interval of at least one week. The large variations in the figures obtained in different groups of one species and in different blood

TABLE I.
Astacus trowbridgii.

	Freezing point depression	Ca mg./cc.	K mg./cc.	Mg mg./cc.
Group I	.79	.43	.23	.066
	.81	.48	—	.072
	.80	.45	.19	.075
Group II	.88	.54	.22	.076
	.84	.45	.16	.077
Group III	.86	.49	.17	.071
	.81	.44	.16	.067

(The figures in Tables I and II are averages of duplicate determinations.)

TABLE II.
Cambarus clarkii.

	Freezing point depression	Ca mg./cc.	K mg./cc.	Mg mg./cc.
Group I	.71	.46	.18	.042
	.72	.44	.16	.044
Group II	.84	.54	.21	.072
	.75	.52	.17	.060
Group III	.82	.55	.19	.069
	.74	.42	.16	.077

⁵ Kramer, B., and Tisdall, F. F., *J. Biol. Chem.*, 1921, **46**, 339; 1921, **47**, 475.

⁶ Clark, E. P., and Collip, J. B., *J. Biol. Chem.*, 1925, **63**, 461.

⁷ Tschopp, E., *Helvetica chimica acta*, 1927, **10**, 843.

⁸ Kuttner, Th., and Cohen, H. R., *J. Biol. Chem.*, 1927, **75**, 517.

samples of the same group have been observed by other authors in the blood of crustaceans and various causes have been suggested. The molting seems to have an important diminishing influence on the molecular concentration of the blood of crustaceans living in freshwater (Bethe and Berger,⁹ Schwabe,¹⁰ Scholles,¹¹ Huf¹²). The production of eggs also influences the composition of the blood very definitely (Schwabe¹⁰). Starvation has been mentioned as causing a lower molecular concentration of the blood. But even without these special conditions the blood of these animals does not seem to be very constant. Huf detected variations in the Cl of the blood of 10% in one specimen of *Astacus fluviatilis* in the course of one to 2 weeks. According to Scholles repetitive bleeding causes only a small diminution of the molecular concentration of the blood of *Eriochier sinensis* living in fresh water. Therefore, one cannot be surprised at the differences in the various figures of the Tables I and II, as no attempt has been made to examine animals living under exactly the same circumstances and at the same periods of their lives. The animals of the first group of both species had lived in a freshwater aquarium and were fed for many weeks before blood was taken for the first time. The first figures of Groups II and III from *Astacus trowbridgii* as well as from *Cambarus clarkii* were obtained from blood samples taken as soon as the animals were brought in. Almost all these figures are higher than those of Group I and higher than the other figures of Groups II and III obtained one week later. This is understandable since all the factors mentioned above as influencing the composition of the blood cause a lowering of the molecular concentration. It seems reasonable to consider the first figures of the Groups II and III of both species as those from animals living most nearly in the normal state. For *Astacus* and for *Cambarus* a freezing point depression of about .85°, and a Ca, K and Mg

TABLE III.
pH of the Blood.

Sample	<i>Cambarus clarkii</i>	<i>Astacus trowbridgii</i>
A	7.54	7.60
B	7.53	7.67
C	7.56	7.41
D	7.54	7.53
E	7.48	
F	7.63	

⁹ Bethe, A., and Berger, E., *Pflügers Arch.*, 1931, **227**, 571.

¹⁰ Schwabe, E., *Z. f. vergl. Physiol.*, 1933, **19**, 183.

¹¹ Scholles, W., *Z. f. vergl. Physiol.*, 1933, **19**, 522.

¹² Huf, E., *Pflügers Arch.*, 1933, **232**, 559.

concentration in the blood of respectively .55, .20 and .07 mg. per cc. must be considered as normal. The pH values, determined on the blood of these 2 species are collected in Table III. A pH of 7.55 can be taken as the average value for both species.

It is interesting that the composition of the blood of these 2 American freshwater crayfishes agrees in general with that obtained from *Astacus fluviatilis* studied in Europe (Berger,¹³ Scholles).

A physiological salt solution based on the figures given above was prepared; containing 12 gm. sodium chloride, 1.5 gm. calcium chloride, .4 gm. potassium chloride, .25 gm. magnesium chloride, and .2 gm. sodium bicarbonate per liter.

The survival of the nerve in this solution was investigated. The nerve bundle in the meropodite of the claw was prepared, and the preparation bathed in the solution. At regular intervals the nerve was stimulated faradically. The contraction of the adductor muscle of the claw was taken as an indicator of the irritability of the preparation. As the muscles in the propodite remain in the shell, they are not exposed to the solution in which the preparation is lying, so the influence of the solution on the nerve only was investigated. After about 15 minutes' immersion, the threshold of the stimulation was found. The irritability of the nerve was then examined at regular times. In general the threshold stayed at the same level for many hours, then dropped rather suddenly. The time was estimated from the moment the preparation was immersed in the solution until the nerve needed a stimulus so strong that the part of it situated in the carpopodite, not exposed to the solution, was stimulated by current escape. The experiments were carried out in a constant temperature room at a temperature between 17 and 18°.

Of 12 preparations of *Cambarus clarkii*, bathed in the physiological solution, the average time during which the nerve kept its irritability was 26 hours; one preparation of this group remained irritable as long as 32 hours. For 12 preparations of *Astacus trowbridgii* these periods were 25 and 35 hours respectively. The same experiments were done with other solutions, namely an isotonic sodium chloride solution and the solution used by Segaar. The nerve in an isotonic salt solution remained irritable only for a short time. The average time for *Cambarus* was 50 minutes; the longest time 70 minutes. It is probable that the nerves of the crayfish bathed in an isotonic salt solution lose their irritability so soon because of the absence of the relatively thick layer of myeline

¹³ Berger, E., *Pflügers Arch.*, 1931, **228**, 790.

which in the frog nerve covers the nerve fibre. The solution of twice the ordinary strength, as the Ringer solution for the frog, was not as ineffective as would be expected from the description of Segaar. Using 12 preparations the average period of irritability in this solution was 6 hours; the longest 11 hours.

Adding Ca, K and Mg ions changes an isotonic sodium chloride solution, in which the crayfish nerve kept its irritability for only 50 minutes on the average, to solution in which the irritability is kept for 26 hours. Is it necessary to add all these ions to obtain this result or may some of them be left out? It turned out that it is particularly the Ca ion that improves the isotonic salt solution. Adding the proper amount of calcium chloride (1.5 gm. per liter) to the isotonic solution gives a fluid in which the nerve kept its irritability for an average of 21.5 hours; the longest time was 28 hours. Adding potassium and magnesium chloride in the right amount (.4 gm. and .25 gm. per liter respectively) did not improve the isotonic salt solution very much. The nerves of 12 preparations stayed irritable for an average of 95 minutes; the longest time was 160 minutes. As the first solution ($\text{NaCl} + \text{CaCl}_2$) maintained the irritability nearly as long as did the complete physiological solution, and as the second solution ($\text{NaCl} + \text{KCl} + \text{MgCl}_2$) maintained it only slightly longer than the isotonic salt solution, it is clear that the calcium ion is one of the important ions for maintaining the irritability of the nerve in crustaceans. However, some influence of the other ions (K or Mg or both) cannot be denied. The fact that the double Ringer solution keeps the irritability of the nerve for an average of 6 hours must be ascribed to the calcium chloride content of .22 gm. per liter, though this is only $1/7$ of the amount in the physiological solution described above.

8648 P

A Study of the Cardia in Unanesthetized Dogs.*

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Gastric and œsophageal fistulæ have been made in 15 dogs for the purpose of studying the activity of the cardia and stomach following stimulation of the œsophagus. For recording changes in tonus a small balloon about 3 cm. long containing rubber sponge with a diameter of 0.5 to 1 cm. was used in the cardia and a large balloon in the stomach. The balloon in the cardia was attached to a fairly rigid tube such that it could be held in place by the hand. It was attached to a tambour and the stomach balloon to a chloroform manometer for recording. A third balloon was placed in the œsophageal fistula well above the cardia. When a stimulus was desired the operator blew into the tube to this balloon distending it. It thus acted as a lodged bolus that could be called into play and allowed to disappear at will. Dogs were trained to lie quietly while tracings were being taken and no anesthesia was used.

Fig. 1 shows the response following distension of the upper bal-

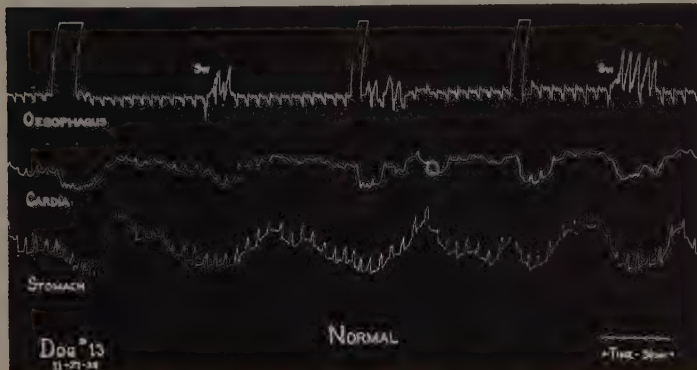


FIG. 1.

Upper tracing from balloon in œsophagus. Effect of distending this upon cardia and stomach shown. "sw", effect of swallowing.

*Aided by a grant from the Committee on Scientific Research of the American Medical Association.

loon in the œsophagus in an animal without nerve section. A relaxation of the cardia and cardiac portion of the stomach (receptive relaxation) takes place immediately. Cutting one vagus did not alter this inhibitory response in the cardia and stomach. In 3 dogs section of both vagi above the diaphragm failed to produce any recognizable spasm of the cardia nor did it cause a material alteration in the inhibition seen in the cardia and stomach following distension of the œsophagus. The response was not affected in 3 dogs with splanchnics cut. Two dogs have had both vagi cut (above the diaphragm) and splanchnics cut. Fig. 2 shows the re-



FIG. 2.

Effect upon this receptive relaxation of sectioning the vagi and splanchnics. The effect upon the tracing of gradually raising the cardia balloon is shown.

sponse in one of these animals. Both animals retained the momentary inhibitory reflex on the cardia and stomach following distension of the œsophagus. This relaxation does not depend upon the initiation of a secondary peristaltic wave in the œsophagus which passes to the cardia but may be brought about by a stimulus not strong enough to produce a peristaltic wave.

These observations indicate that relaxation of the cardia and cardiac portion of the stomach following a peristaltic wave of the œsophagus is not dependent upon extrinsic nerves.

8649 P

Enhancement of *H. pertussis* Virulence with Starch and Use of Starch Cultures in Mouse Immunity Tests.

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We have dealt¹ with the enhancement of virulence of meningococci and typhoid bacilli for mice, and the effect of both active and passive immunization upon resistance of mice to infection with mucin-coated cultures. This work followed that of Nungester, Wolf and Jourdonais,² Miller³ and Rake,⁴ and in turn was followed by a similar report by Mishulow and Melman.⁵ These reports show that the intraperitoneal mouse-virulence of certain strains of meningococci and typhoid bacilli may be multiplied a thousand- to a million-fold through the use of the proper gastric mucin, and that this procedure may be of use in the selection of bacterial vaccine strains and in turn in the evaluation of antiserum.

Subsequently we have found the "mucin-coating" technic applicable to *B. coli*, gonococci, and *Streptococcus viridans*.⁶ In the following experiments we applied similar methods of virulence-enhancement to *H. pertussis*.

We have utilized 41 freshly isolated smooth strains, as described by Shibley and Hoelscher,⁷ which fulfill Phase 1 characteristics as stated by Leslie and Gardner.⁸ Twenty-four-hour growths on Bordet-agar slants enriched with 25% human or sheep blood, planted from the second of a series of two 48-hour transplants, were washed off with 1 cc. of infusion broth and further dilutions prepared from this as indicated.

Mucin-virulence: The mucin-coating technic has been applied with several minor variations to 36 pertussis cultures injected into

¹ Henderson, R., Jamieson, W. A., and Powell, H. M., *Proc. Ind. Acad. Science*, in press.

² Nungester, W. J., Wolfe, A. A., and Jourdonais, L. F., *Proc. Soc. Exp. Biol. and Med.*, 1932, **30**, 120.

³ Miller, C. P., *Science*, 1933, **78**, 340; *Proc. Soc. Exp. Biol. and Med.*, 1935, **32**, 1136, 1138, 1140.

⁴ Rake, G., *J. Exp. Med.*, 1935, **61**, 545; *Proc. Soc. Exp. Biol. and Med.*, 1935, **32**, 1175, 1523.

⁵ Mishulow, L., and Melman, M., *J. Lab. and Clin. Med.*, 1936, **21**, 406.

⁶ Unpublished experiments.

⁷ Shibley, G. S., and Hoelscher, H., *J. Exp. Med.*, 1934, **60**, 403.

⁸ Leslie, P. H., and Gardner, A. D., *J. Hyg.*, 1931, **31**, 423.

530 mice. None of the changes in technic affected the outcome of the tests. Only an occasional mouse died, and these few deaths bore little or no relation to the dose of mucin-coated culture injected. Similar results were obtained in 2 series of tests in 12 guinea pigs. Obviously mucin is of relatively little use in attempts to enhance the virulence of *H. pertussis*.

Starch-virulence: Of 9 different grades of starch† obtained from the Corn Products Refining Company through the courtesy of Mr. R. H. DeWaters, the starch called "145-60 thick boiling starch" prepared in a 4% solution with 0.5% glycerin proved most useful in coating *H. pertussis* with resultant increase in virulence for mice. The results of several tests of 6 different cultures with starch are shown in Table I. A large percentage of mice were

TABLE I.
Range of Mouse-Virulence of Several Pertussis Cultures Coated with Starch at Different Times.

Culture dose (cc.)	No. of mice	
	Died	Lived
10-1	42	0
10-2	33	9
10-3	14	27
10-4	10	31
10-5	0	11

killed with doses of 10^{-4} cc. Two quantitative active immunity tests in mice were tried.

Active immunity in mice: 12 mice received 3 weekly subcutaneous injections each of Pertussis Vaccine (Sauer) lot 921499 Lilly; 12 more received Pertussis Undenatured Bacterial Antigen (Krueger), lot 915013 Lilly. Two weeks after the last dose, 5 mice of each group and 5 controls were given intraperitoneal injections of decimal dilutions of starch-coated culture. The virulence of the test culture at this time only reached 10^{-2} cc.; however, both groups of vaccinated mice were protected against one or more fatal doses. These results are shown in test number 1 in Table II.

†No. 55 Hercules gum thin boiling starch.
No. 106 Alkali starch.
No. 107 " "
No. 126 Thick boiling starch.
No. 131 Thin " "
No. 140 " " "
No. 142 Thick " "
No. 145-30 " " "
No. 145-60 " " "

TABLE II.
Tests of Active Immunity of Mice After Immunization Against Pertussis.

Immunity-test dose of starch-coated <i>H. pertussis</i> (in cc.)	Pertussis Vaccine (Sauer) No. 921499 Mice		Pertussis Undenatured Bacterial Antigen (Krueger) No. 915013 Mice		Control Mice	
	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
10-1	D	DD	D	DD	D	DD
10-2	S	DD	S	DS	D	DD
10-3	S	S	S	S	S	D
10-4	S	S	S	S	S	D
10-5	S	S	S	S	S	S

D—A mouse dead within a week.

S—A mouse living after a week.

Four weeks after the last dose of antigen, the remaining 7 mice in each group, and 7 controls, were given an immunity test as before. The virulence of the test culture at this time was 10^{-4} cc. Both groups of vaccinated mice exhibited protection against 10 or more fatal doses. These results are shown in test number 2 in Table II. It is obvious that additional tests should be conducted for exact quantitative measurements, however the procedure appears to be of use in view of the meager laboratory criteria of virulence and antigenicity of *H. pertussis*.

8650 C

Vitamin G from Different Sources and Coccidian Infection.*

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The writers^{1, 2} have previously shown that a diet deficient in vitamin G has a pronounced limiting effect on the numbers of oocysts discharged by rats infected with the coccidium *Eimeria miyairii*. The present work was undertaken to ascertain the effect of vitamin G from sources other than yeast.

Experiment I. Thirteen rats were kept for 14 days on a balanced growing ration (Steenbock's) containing corn meal, linseed

* Supported in part by a grant from the Industrial Science Research Fund of Iowa State College.

¹ Becker, E. R., and Morehouse, N. F., *Proc. Soc. Exp. Biol. and Med.*, 1935, **33**, 114.

² Becker, E. R., and Morehouse, N. F., *ibid.*, 1936, **33**, 487.

oil meal, casein, alfalfa, skim milk and salt. Subsequent to infection with 1500 oocysts of *E. miyairii* daily for 5 days this lot eliminated oocysts as follows, in terms of ten millions: 23, 28, 20, 19, 23, 13, 16, 27, 30, 17, 29, 14, 36; mean, 22.69. Thirteen rats were given the basal ration² made up to 10% with powdered yeast. These eliminated ten millions of oocysts as follows: 14, 24, 32, 18, 14, 12, 22, 34, 31, 23, 32, 17, 18; mean, 22.385. The coccidium-growth promoting factor in yeast is hereby shown to be present likewise in other materials, for the analysis of variance test³ showed the data of the 2 series not to be significantly different.

Experiment II. The following low-G ration was prepared: white corn meal, 25; beet sugar, 48; wheat gluten, 10; casein, acid-water extracted, 8; salt mixture, 2; butter, 5; cod liver oil, 2. The reference diet was the growing ration. In the first trial 7 rats on the low-G diet for 2 weeks discharged oocysts as follows, in millions, after infection: 48, 91, 165, 95, 94, 34, 117; mean, 92. The same for reference series: 301, 144, 273, 153, 180, 231, 419; mean, 243. In the second trial: low-G series,—62, 82, 32, 63, 59, 37, 61, 65; mean, 57.62; reference series,—194, 128, 121, 35, 204, 88, 206; mean, 139.43. It is evident that the diet low in vitamin G exerted a marked limiting effect on the development of the coccidium oocysts. Also, the analysis of variance test indicated that the data from the two series, *i. e.*, low G and normal, were significantly different.

Experiment III. With the first trial of the preceding experiment were 7 rats on the low-G diet, supplemented with 8% autoclaved yeast 3 days before infection. These eliminated 192, 107, 176, 131, 163, 129, and 154 millions of oocysts; mean, 150.29. The mean is significantly different (Fisher's⁴ small sample method) from the mean of 92 million for the low-G diet, showing that the autoclaved yeast was the differential factor.

Experiment IV. With the second trial of *Experiment II* were 9 rats on the basal ration² supplemented with 10% wheat germ as a source of vitamins B and G. These discharged millions of oocysts as follows: 85, 20, 100, 122, 90, 134, 69, 140, and 148; mean, 100.89. This mean is not significantly different from that of 139.43 for the rats on the growing ration, but is significantly from that of 57.62 for the low-G series. Wheat germ, then, is a source of the coccidium-growth promoting substance.

³ Snedecor, G. W., *Analysis of Variance*, 1934.

⁴ Fisher, R. A., *Statistical Methods for Research Workers*, 1930.

Experiment V. An alcoholic pork liver extract equivalent to 4 gm. of liver per cc. was prepared by the method of Lepkovsky, Popper and Evans.⁵ *First trial.* Five rats received the basal diet,² with one cc. liver extract dried onto the sugar each day. After infection these eliminated oocysts in millions as follows: 58, 82, 72, 86, 71; mean, 73.8. The controls receiving the basal ration² plus 10% yeast: 260, 289, 214, 249, and 355; mean 273.4. *Second trial.* The recipients of the liver extract discharged by individuals millions of oocysts as follows: 36, 27, 61, 9, 22; mean 31. The yeast recipients: 156, 110, 135, 128, 186; mean, 143. In both trials the animals were on the diet 11 days before infection began. The means in both cases are significantly different, indicating that the liver extract did not contain the coccidium-growth promoting factor.

Experiment VI. Five rats received for 11 days the basal ration made up to 2% with dried and powdered pork liver and up to 2.5% with rice polish. After infection they eliminated by individuals 52, 5, 29, 30, and 40 million oocysts; mean, 31.2. The reference series on the basal diet² made up to 10% with powdered yeast: 111, 222, 236, 215, 462; mean, 249.2. This experiment likewise shows that liver as a source of vitamin G does not stimulate the development of the coccidia. It should be stated that the differences are statistically significant when tested by Fisher's⁴ small sample method, although the numbers are small.

The data submitted here indicate conclusively that coccidium growth is tied up with the vitamin-G complex. When yeast or certain other materials are employed as a source of this vitamin, growth of the parasite is stimulated. Liver as a source of the vitamin does not favor the coccidium. The method whereby the coccidium-growth promoting factor affects the parasite is at present undetermined.

⁵ Lepkovsky, S., Popper, W., and Evans, H. M., *J. Biol. Chem.*, 1935, **109**, liv.

Isohemolysins and Isoagglutinins Occurring in Dogs.

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Plasmapheresis as used in this laboratory means the removal of blood cells and plasma by bleeding followed at once by the replacement of normal red cells suspended in a modified Locke's solution. In this way plasma depletion is readily accomplished and the regeneration of new plasma protein as influenced by various factors can be studied.¹ In the course of such experiments many unfortunate developments may complicate the picture and obscure results. Among these complications *hemolysis* has been noted all too frequently in certain dogs following exchanges. These dogs became sick with vomiting, diarrhœa, hemoglobinuria, jaundice, anemia and even severe shock. Other dogs, however, have received similar exchanges of red cells from 3 to 6 times a week for long periods of time from the same group of 11 donor dogs without untoward effect.

It is known that there are indistinct differences of blood types in dogs.² The rarity of occurrence of these differences and difficulty in their demonstration have made consideration of blood grouping in dogs unnecessary for ordinary experimental procedures. Isohemolysins and isoagglutinins in dogs following repeated direct transfusions have been reported.³ Recently, Melnick, Burack and Cowgill⁴ have reported incompatibilities occurring in dogs on plasmapheresis experiments, similar to those noted in this laboratory. Baldrige⁵ noted similar changes resulting in the death of one dog which had previously received several transfusions from an originally compatible donor.

Determination of the results following the *in vitro* mixture of the sera of the various recipient dogs with the red cells of the

¹ McNaught, J. B., Scott, V. C., Woods, F. M., and Whipple, G. H., *J. Exp. Med.*, 1936, **63**, 277.

² Wiener, A. S., *Blood Groups and Blood Transfusion*, Springfield, Ill., Charles C. Thomas, 1935.

³ Ottenberg, R., Kaliski, D. J., and Friedman, S. S., *J. Med. Res.*, 1913, **28**, 141.

⁴ Melnick, D., Burack, E., and Cowgill, G. R., *Proc. Soc. Exp. Biol. and Med.*, 1936, **33**, 616.

⁵ Baldrige, C. W., *Arch. Int. Med.*, 1934, **54**, 517.

donor group were made. Observations were made of both macroscopic and microscopic hemolysis and agglutination.

The procedure for the macroscopic method consisted of mixing in a test tube (7 mm. inner diameter) 0.25 cc. of the clear unhemolyzed recipient serum with 0.5 cc. of a 5% suspension of fresh, washed red cells from the donor to be tested. The mixture was then incubated at 37°C. for 2 hours with observation at half hour intervals. Hemolysis was indicated by change of the cloudy cell suspension to a clear cherry-red color. Agglutination occurred early as indicated by marked clumping of the red cells at the bottom of the tube in distinct contrast to the controls. The microscopic tests were made by use of hanging drop preparations.

The first group of dogs consists of 3 animals which were used in plasmapheresis experiments until development of the abnormalities described was so severe as to preclude their further use.

Dog 34-174, a female mongrel hound, was used in a plasmapheresis experiment for a period of over one month, and received in this time 22 red cell exchanges. The animal then developed marked hemolysis of the red cells, became jaundiced, was weak, feverish and refused food following injection of red cells. The experiment was then terminated and the dog was not used for a period of 8 months. The dog's serum was then tested *in vitro* against the red cells of the group of 11 donor dogs. The red cells of 5 of the donor group were hemolyzed and agglutinated while the remaining 6 were compatible. The dog was then given intravenously, without reaction, 15 cc. of washed, packed red cells, suspended in an equal quantity of Locke's solution, from a compatible donor dog. Two days later, an injection of 5 cc. of similarly prepared red cells from another compatible donor was given, and on the next day, 6 cc. from another compatible donor. There was no evidence of reaction following any of these injections. After this the red cells of 2 of these donors, previously compatible by both *in vitro* and *in vivo* test, were found to be incompatible *in vitro* as evidenced by hemolysis and agglutination.

Dog 34-180, a female mongrel terrier, first developed evidence of hemolysis of red cells after it had received 4 red cell exchanges in a period of 10 days. With following injections of red cells there was increased hemolysis, hemoglobinuria, diarrhoea. In all, 12 exchanges of red cells were made over a period of 20 days when the experiment was terminated. After a period of 8 months' rest the serum of this dog was tested *in vitro* with the red cells of the group of 11 donor dogs. The red cells of 7 of the donors were

hemolyzed and agglutinated while the red cells of the remaining 4 showed no evidence of incompatibility.

Dog 34-53, a mongrel male poodle, was used in plasmapheresis experiments for a period of over one month, and received 31 red cell exchanges before similar abnormalities were noted and the experiment was terminated. After a period of 9 months, this dog was given 3 red cell exchanges. Following each injection, the dog developed hemolysis of red cells, hemoglobinuria, vomiting and diarrhoea. After a period of 3 more months this dog was given several intravenous injections of red cells from donors which were incompatible by *in vitro* test. Severe reactions marked by the signs described previously immediately followed each injection. The dog was then given without reaction, intravenous injections of red cells from 2 dogs compatible by *in vitro* test. Several days later, the red cells of these 2 previously compatible dogs were markedly incompatible as indicated *in vitro* by hemolysis and agglutination. At this time, the serum of this dog tested against the red cells of the donor group, rapidly hemolyzed and agglutinated the red cells of 7 of these dogs while 4 were compatible.

The red cells of the above 3 plasmapheresis recipient dogs, tested *in vitro* with the sera of the group of donor dogs, showed no evidence of incompatibility.

In contrast to the difficulties experienced with the 3 dogs discussed above, many plasmapheresis experiments have been performed in this laboratory in recent years without development of such abnormalities in the recipient dogs. The following 2 dogs illustrate the frequently repeated, successful use of dogs in plasmapheresis. *Dog 33-11*, a female mongrel hound, was used for a period of over 5 months, during which time it received 110 red cell exchanges from the same donor group without evidence of reaction. *Dog 34-152*, a male mongrel hound, was used for a period of over 9 months and received 190 red cell exchanges without reaction.

The sera of these 2 animals showed no evidence of incompatibility with the red cells of the donor group in hanging drop preparations. However, in the macroscopic procedure, mixture of the sera of these 2 animals with the red cells of the donor dogs, showed hemolysis which was not evident in controls with the sera of normal dogs. In these observations there was no evidence of macroscopic agglutination, nor was hemolysis as marked as when such tests were made with the sera of the 3 plasmapheresis dogs first discussed.

Because the red cells of some of the donor dogs were hemolyzed directly *in vitro* without evidence of agglutination, it was desirable to determine if there was fixation of complement in this type of hemolysis. Considerable difficulty was experienced in the use of an isohemolytic system. This has also been the experience of others working with isohemolysins.² Inactivation of the complement in these sera by heating at 55°C. for 30 minutes caused a reduction of hemolysis of from one-half to two-thirds of that seen in the active controls.

Conclusions. Isohemolytic and isoagglutinative phenomena noted in occasional dogs during plasmapheresis, appear to be related to antigenic formation of immune bodies by the recipient due to certain factors in the donors' red cells. These hemolytic and agglutinative properties persist in the serum for long periods of time and may be intensified by renewed injection of red cells. Isohemolysis of the red cells of dogs appears to depend at least in part upon fixation of complement.

8652 C

A Purified Diet Satisfactory for Growth, Reproduction and Lactation in Rats.

WARREN M. COX, JR. AND MIRIAM IMBODEN.

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The majority of investigative work on the adequacy or inadequacy of a given dietary regimen has involved principally 2 criteria: the rate of growth and the maximum adult weight attained. Osborne and Mendel¹ showed, on this basis, the improvement that had been accomplished in stock diets when growth records were compared with Donaldson's standards for the albino rat.² More recently, Bills' modification of the Steenbock diet* has been widely employed, and we have recently compared reproductive behavior

¹ Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1926, **69**, 661.

² Donaldson, H. H., *Wistar Institute of Anatomy and Biology*, Philadelphia, 1924, p. 275.

* Composition: Yellow maize, 57; whole milk powder, 25; linseed oil meal, 12; crude casein, 3.7; alfalfa leaf meal, 1.5; "iodized" table salt, 0.4; calcium carbonate, 0.4 %.

on this stock diet with that obtained with a purified diet containing various amounts of calcium and phosphorus.³

Some attempt has been made to devise diets of purified food-stuffs that will compare favorably, *over several generations*, with stock diets.⁴ The results were not eminently successful.

In the work reported elsewhere³ we fed a purified diet during the whole reproductive life of rat mothers. In order to determine the usefulness of this purified diet, we have fed it as the sole food for a period of more than 2 years, to 3 generations of rats.

EXPERIMENTAL.

The composition of the diet is:

Casein, washed repeatedly with dil. acetic acid	20.0
Dextrin, from corn starch by heating with citric acid	55.1
Lard, Swift's Silverleaf	9.0
Aqueous extract brewer's yeast, purified from protein, and dried (equiv. in B ₁ to 24%, in B ₂ to 8% yeast)	4.0
Wheat Germ Oil	1.6
Carotene Solution 3:1000, in corn oil	0.3
Calcium Acetate	1.0
Salts†	3.1
Rice Cellulose	5.9

The components of the dry diet were mixed not less often than every 2 weeks, and stored at 50°F. The diet was supplied, (as was also distilled water) *ad libitum*.

Five young stock females (200 gm.) were allowed to raise 3 consecutive litters (each reduced to six pups at birth) to 24 days of age. Weights were recorded (and are reported) at 21 days of age, for comparison with our earlier work. The first 2 litters were raised to 100 days of age, and mated to males similarly raised. The 2 groups of 3 consecutive litters which resulted were treated as was the first generation;—their young constituting the third generation.

In each instance the third litter was killed, and the carcasses ashed at 550°C. in an electric muffle.

The reproductive records of one group of stock controls, and of 3 generations of animals on our purified diet are given in Table I. It should be noted that in order to obtain an average growth curve for stock females we made 653 weighings of rats of varying ages,

³ Cox, W. M., Jr., and Imboden, M., *J. Nutrition*, 1936, **11**, 147.

⁴ Hartwell, G. A., *Biochem. J.*, 1926, **20**, 1273; 1927, **21**, 1076.

† Composition: NaCl, 24.50; MgSO₄, 9.14; KHCO₃, 59.20; KCl, 3.84; Fe citrate, 2.94; CuSO₄, 0.32; MnSO₄, 0.04; KI, 0.02%.

TABLE I.
Reproduction Records of Stock Controls, and Experimental Animals Receiving a Diet of Purified Foodstuffs.

Diet	Gestation No.	No. Females	Wt. at First Mating	No. Young Born	No. Avail-able to Raise	% Raised	Av. Wt. 21 days gn.	% Ash	Wt. Gain of Mothers in cycle gn.	Total Gain over Initial Wt.		Wt. of offspring at 100 days of age	
										♂	♀	♂	♀
Stock	1	10	96.9*	98	60	100	39.2		41.0				
	2	10	192.2	93	57	96.4	44.5		40.7				
	3	10		107	60	86.6	45.5	2.70	22.9	103.3			193.0†
	Av.			9.9	5.9	94.4	42.9		34.9				
Purified Diet 1st Generation	1	5	98.2*	55	31	100	37.6		37.2				
	2	5	205.0	46	27	100	44.1		30.0				
	3	5		49	30	100	46.7	2.74	33.5	94.6			
	Av.			10.0	5.9	100	42.7		33.6				
Purified Diet 2nd Generation	1	9	100.8*	94	54	100	41.3		42.8				
	2	9	202.7	82	53	94.3	42.8		29.0				
	3	9		91	54	85.2	46.3	2.61	25.4	77.3			
	Av.			9.9	6.0	93.2	43.2		32.3				
Purified Diet 3rd Generation	1	19	99.8*	140	90	78.9	41.8		34.0				
	2	19	195.9	161	100	89.0	42.5		25.2				
	3	19		150	92	90.2	44.2	2.68	26.3	83.2			
	Av.			8.8	5.5	86.2	42.9		28.6				

*Age in days at first mating.

†Computed from growth records of 66 stock female rats.

—all housed in our stock rattery, and saved for breeding purposes. Only the larger rats (at 24 days of age) are saved for breeding.

From Table I it is evident that reproduction and lactation on a purified diet can be the equal of that on a mixed stock diet. Commonly, 3 criteria may be employed to determine the adequacy of diets:—the weight of nurslings at 21 days of age, the gain in weight of the mother, and the ash content of the young.³ For the stock controls and experimental animals, first, second, and third generations, respectively, we have: weight of young at 21 days,—42.9, 42.7, 43.2 and 42.9 gm.; final adult weight of the mothers,—295.5, 299.6, 280.0 and 279.1 gm.; gross body ash,—2.70, 2.74, 2.61 and 2.68%. The differences are so small that we can attribute no significance to them.

From the standpoint of reproduction there appears to be a small decrease in the number of young born in the third generation: the controls averaged 9.9 pups per mother per litter, while the third generation averaged but 8.8 per litter. Many factors determine the number of young born, but, in the present experiment, as all young born were living, and of average weight at birth, we cannot attribute much importance to the observed difference.

The percentage of young raised showed a small decrease in the third generation, as compared with the controls—in spite of the fact that the average weight of nurslings at 21 days was the same. We are inclined to discount this small difference in view of the fact that most of the young in this generation were born in the months May to September, whereas the 3 control litters were born October to February.

Table I gives the weights of the 3 generations of males and females on the experimental diet, at 100 days of age as compared with the average weight of stock females. It is evident that there is no difference between adult weights of these 3 generations of experimental rats, when compared with the weight of stock females.

Summary. Three generations of rats have been raised to maturity while receiving a diet composed solely of purified foodstuffs. It is shown that growth, reproduction and lactation on this diet are as satisfactory in every way as when a standard stock diet is employed.

8653 P

Formation of Intra-Ocular Fluid and Permeability of the Ciliary Body.*

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From the Wilmer Ophthalmological Institute, Johns Hopkins University Medical School.

Previous investigations have shown that there is a continuous circulation of the intra-ocular fluid, the chief source being the ciliary body, the chief exit being the canal of Schlemm.¹ The ciliary body exhibits an irreciprocal permeability to water and to methylene blue, both of which pass readily from stroma to epithelium but not in the reverse direction.² The present study was directed toward the discovery of the physico-chemical factors involved in this irreciprocal permeability. We have found that the tissue is permeable in both directions to acid and neutral crystalloid dyes, but impermeable in both directions to non-reducible basic crystalloid dyes. The stroma is capable of reducing methylene blue while the epithelium is capable of oxidizing methylene white. The passage of methylene blue from stroma to epithelium occurs therefore in its reduced, neutral state. By using a series of oxidation-reduction indicators, we found that the difference in oxidation-reduction potential between the epithelium and the stroma amounts to 100 millivolts.

At a pH of 5.3-5.5 the charge on the membrane barrier between epithelium and stroma is reversed, at lower pH the membrane barrier is permeable to basic dyes, impermeable to acid dyes, at higher pH the reverse is true. The membrane, therefore, is negatively charged at normal pH. This conclusion was confirmed by the production of an artificial electro-endosmotic flow in the proper direction through the tissue by means of an externally applied E.M.F.

In order that there should be a unidirectional flow of water by electro-endosmosis through a charged membrane there must be an electron conductor in the system. It is possible that a reversible oxidation-reduction system within the membrane could act as such an electron conductor. If the charge on the membrane is reversibly

* The details of this study will be published in the *Archives of Ophthalmology*.

¹ *Arch. of Ophth.*, 1932, **7**, 538; 1932, **8**, 9.

² *Trans. Amer. Ophthal. Soc.*, 1933, **31**, 143.

altered by the addition of oxidants or reductants at a fixed pH, the membrane must contain a reversible oxidation-reduction system. By operating at a pH of 5.7 we were able to reverse the charge on the membrane by the addition of oxidants, and to regain the normal charge by subsequent reduction. *In summary*, the physiological flow of water from stroma to epithelium, *i. e.*, from blood capillaries to intra-ocular cavity, is the result of electro-endosmosis. The mechanism for this electro-endosmosis is a difference in oxidation-reduction potential across a charged membrane. It is postulated that the reversible oxidation-reduction system within the membrane acts as the electron conductor necessary to complete the electrical circuit.

8654 P

Differentiation of Pigment Cells in Tissue Cultures of Chick Neural Crest.

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The experiments of DuShane^{1, 2} have proved conclusively that the dermal melanophores of the amphibia originate in the neural crest, as suggested by Harrison,³ and later by Weidenreich⁴ and Holtfreter.⁵ The differentiation of large numbers of typical pigment cells in tissue cultures of neural crest taken from embryos of pigmented fowls indicates the probability of a similar source of origin in the chick.

Embryos of various breeds have been used, ranging in stage between 6 and 10 somites. Thin strips of the rising folds anterior to the first somites were removed with fine scissors and explanted in a medium consisting of equal parts of plasma and embryonic extract. For controls, whole embryos minus the neural crest were cut into small pieces and explanted on a single slide; or specific areas, such as the eye, the brain floor with underlying endoderm,

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¹ DuShane, G. P., *Anat. Rec.*, 1934, **60**, 62.

² DuShane, G. P., *J. Exp. Zool.*, 1935, **72**, 1.

³ Harrison, R. G., *J. Exp. Zool.*, 1910, **9**, 787.

⁴ Weidenreich, F., *Z. f. Morphol. u. Anthropol.*, **2**, 59.

⁵ Holtfreter, J., *Roux' Arch.*, 1933, **127**, 619.

and the ectoderm and mesoderm lateral to the crest region, were explanted separately. Control cultures never contained more than one or 2 isolated pigment cells, and were for the most part totally negative.

The cells appear at the border of the explant during the first day *in vitro*. They are extremely variable in shape, ranging from bipolar to stellate forms, but are frequently found with long, thin, branching processes. They are filled with colorless, refractile granules, which gradually acquire pigment, developing from a pale yellow to deep brown or black, and reaching full intensity in many cells by the third day *in vitro*. The cells are actively amoeboid, and show no tendency to form sheets or tissues, a trait which distinguishes them sharply from the pigmented epithelium of the eye, and from the nervous tissue and epithelium which frequently make up the remainder of such explants.

Pigmented cells of similar appearance and behavior were obtained from explants of limb buds of 3-day embryos of the Silky, a fowl having much pigment in the connective tissue, while Koller⁶ found them in cultures of trunk mesenchyme from 3 and 4-day Black Leghorn embryos. Since no pigmented cells appear in cultures of mesenchyme taken from such embryos in early somite stages, it seems reasonable to assume that the cells found in the mesenchyme of later stages have wandered out from their source of origin in the neural crest.

The present experiments may throw some light on the genetic situation with regard to pigmentation in fowls. There are 2 types of whiteness in fowls,⁷ a dominant white, in which the presence of the color factor is masked by a coexisting inhibitor; and a recessive white, lacking both color factor and inhibitor. Cultures of the neural crest of the Black Minorca and the Barred Plymouth Rock, both dominant blacks, invariably produced pigmented cells. Cultures of the Rhode Island Red, a non-melanotic breed, and of the White Plymouth Rock, a recessive white, show no trace of these cells, developing usually into nervous tissue, epithelium, and indifferent fibroblasts.

⁶ Koller, P. C., *Arch. f. Exp. Zellforsch.*, 1929, **7-8**, 491.

⁷ Sinnott, E. W., and Dunn, L. C., *Principles of Genetics*, 1932, **83**.

Studies on Sodium Loss.*

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The method devised by Schechter, Cary, Carpentieri, and Darrow¹ was used to produce sodium insufficiency. Usually a liter of an isotonic solution of glucose (5%) was injected aseptically into the peritoneal cavity of fasting dogs. Portions of this glucose solution removed from the peritoneal cavity from one to 2 hours later were analyzed for sodium and chloride. In an effort to make the sodium loss less acute and therefore comparable to that which occurs in Addison's disease, the fluid was injected and then withdrawn each day over a period extending from 4 to 7 days. Such a lapse of time permits daily analysis of the blood and urine as well as observation of the general condition of the animal. The blood was analyzed for sodium,² chlorides,³ and urea.⁴ The creatine and creatinine content of the urine was determined by the method of Folin.⁵

Observations were made on 5 dogs and results were similar in every animal. In Table I is presented a typical experiment. The data disclose a gradual fall of sodium and chloride of the serum, while the solids of the serum and the blood urea increase. These changes are the results of the withdrawal of the electrolytes from the animals as depicted in the last 2 columns.

The urine was analyzed for creatine on the fourth day of withdrawal of electrolytes and a marked creatinuria was observed. This creatinuria is probably not a result of the brief period during which food was withheld from the dogs. Moreover, these animals were absorbing as much as 35 gm. of the glucose injected intraperitoneally daily. It is probable that the creatinuria was associated with the asthenia which developed in these animals.

*This work was aided by grants from the National Research Council and from Schieffelin and Company, New York.

¹ Schechter, A. J., Cary, M. K., Carpentieri, A. L., and Darrow, D. C., *Am. J. Dis. Child.*, 1933 **46**, 1015.

² Hald, P. M., *J. Biol. Chem.*, 1933, **103**, 477.

³ Eisenman, A. J., *J. Biol. Chem.*, 1929, **82**, 411.

⁴ Folin, O., and Wu, H., *J. Biol. Chem.*, 1919, **38**, 81.

⁵ Folin, O., *J. Biol. Chem.*, 1914, **17**, 469.

TABLE I.
Studies on Sodium Loss.

March, 1935	Temp. C	Serum			Blood Urea mg./100 cc.	Fluid withdrawn from Peritoneal Cavity	
		Na m. eq.	Cl m. eq.	Solids gm./l cc.		Na m. eq. per kilo Body Wt.	Cl m. eq. per kilo Body Wt.
11		137.7	109.2	—	8.1	—	—
12		141.8	111.4	.0738	13.5	4.0	4.7
13	38.6	137.5	100.2	.0801	9.6	5.0	5.9
14	38.2	125.8	88.4	.0935	28.7	2.0	2.4
15	38.2	113.5	82.8	.0910	51.0	2.0	2.2
16	38.1	105.2	74.2	.0916	72.0	2.4	2.7
Total						15.4	17.9

Weight, March 11—14.1 Kg.

March 16—11.8 "

Urine, 24 hr. sample, March 15

Total Creatinine 699 mg.

Preformed Creatinine 219 "

Creatin 480 "

Summary and Conclusion. The effects of sodium deprivation were observed in 5 dogs over periods varying from 4 to 7 days. Sodium loss produces a condition similar to that of adrenal insufficiency, namely, increased concentration of the blood and a retention of blood urea, loss of serum chlorides and a creatinuria. Sodium loss, however, does not reproduce exactly the same picture as insufficiency of the cortex of the adrenal gland. The present experiments disclose a rise in blood urea concomitant with the depletion of the sodium reserves. In adrenal insufficiency, on the other hand, the increase of blood urea may precede the loss of sodium.⁶ Furthermore, in adrenal insufficiency, the animals usually succumb with a higher level of serum sodium.

8656 C

Antigenic Individuality of Certain Papilloma Viruses.

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New York.*

Papillomas of the skin and mucous membranes are frequent in man and many of the lower animals. That certain of these

⁶Loeb, R. F., Atchley, D. W., Stahl, J., *J. Am. Med. Assn.*, 1935, **104**, 2149.

growths are caused by filterable viruses has been demonstrated, notably verruca vulgaris in man,¹ oral papillomatosis of dogs,² cattle warts³ and the papillomatosis of American cottontail rabbits.⁴ All these growths are readily induced in susceptible hosts by inoculation with the respective viruses. In general, animals of other species than that furnishing the virus prove resistant on inoculation, though some problematic transfers have been reported. The virus of the Shope papilloma of cottontails is undoubtedly effective in other kinds of rabbits,⁵ but guinea pigs, dogs, cats, rats and mice have proved insusceptible. Despite the limitations thus illustrated in the pathogenic range of the viruses, the strikingly similar characters of the growth produced by those deriving from various animals suggest that they may be related, like the viruses causing the pox diseases and the swine and human influenza viruses. We have tested this possibility immunologically, seeking for evidence of cross neutralization between the virus of rabbit papillomatosis and the sera of dogs, rabbits, cattle, and men bearing virus-induced papillomas or recently recovered from them. It is known that these growths induce neutralizing antibodies for the homologous agents with fair regularity. It was impracticable for us to test most of the sera against the homologous agents; and hence we have assumed that they were actively neutralizing.

Serum was procured from a cottontail rabbit trapped in Texas and carrying many cutaneous papillomas, as also from 2 cows of the same Texas neighborhood,—one a yearling heifer bearing characteristic papillomas of the kind due to virus, the other a cow about 2 years old in which similar growths had retrogressed about 4 months previously. As controls, specimens of cattle serum were available from 2 animals in New Jersey, both less than 2 years old, of a herd in which papillomas had never occurred. Two mixtures were made in different proportions of each of the various sera with a freshly prepared Berkefeld V filtrate of an extract of glycerolated warts from a Kansas cottontail rabbit. After incubation for 3 hours at 37° the mixtures, with appropriate con-

¹ Ciuffo, G., *Gior. ital. d. mal. vener.*, 1907, **48**, 12.

² Findlay, G. M., in *A System of Bacteriology in Relation to Medicine*, Great Britain Medical Research Council, London, His Majesty's Stationery Office, 1930, **7**, 252.

³ Magalhaes, O., *Brazil-med.*, 1920, **34**, 430; Creech, G. T., *J. Agric. Research*, 1929, **39**, 723.

⁴ Shope, R. E., *J. Exp. Med.*, 1933, **58**, 607.

⁵ Beard, J. W., and Rous, Peyton, *Proc. Soc. Exp. Biol. and Med.*, 1935, **33**, 191.

trols of Tyrode and virus, were rubbed into small scarified squares on the skin of 3 domestic rabbits, and the squares were kept individually covered until healing had taken place. None of the cow sera had any effect on the virus, the papillomas appearing and growing as well where mixtures containing such sera had been introduced as where the virus-Tyrode mixtures had been inoculated. The serum of the cottontail rabbit on the other hand completely neutralized the virus.

Serum specimens from 2 puppies recently recovered from oral papillomatosis were generously given us by Dr. W. A. DeMonbreun and Dr. E. W. Goodpasture, and specimens were taken from 4 normal puppies, from 2 normal domestic rabbits and 2 carrying large, virus-induced papillomas. Mixtures were made of all with an extract of glycerolated cottontail papilloma which had been cleared by centrifugation; and after incubation, these were inoculated with the appropriate virus-Tyrode control into 8 domestic rabbits in the same way as in the previous experiment. Vigorous growths resulted from every mixture save those containing serum from one or the other of the papillomatous rabbits. The virus had been wholly neutralized by these.

Three persons working in our laboratory and frequently handling rabbit papillomas without the protection of gloves were noted to have multiple small cutaneous papillomas, to all appearance ordinary small warts, on the palms, and in one instance on both ankles as well. It seemed worth while to ascertain whether the blood of these individuals was capable of neutralizing the rabbit virus. Accordingly serum specimens were procured, as also from 2 other workers free from warts and never in contact with the rabbit material. Mixtures were made with rabbit virus and tested as in the experiments already described, by inoculation into 3 rabbits. None of the sera had any neutralizing effect on the virus.

Findlay² has reported that human serum which neutralizes the virus of human warts has no action on that causing oral papillomas in dogs and *vice versa*. His results and ours would appear to demonstrate that there is no antigenic relationship between the viruses responsible for the papillomas of man, dogs and rabbits, nor between those causing papillomas in cattle and rabbits.

Influence of Vagi and Splanchnics upon Blood Sugar Response to Glucose Administration.*

MARK AISNER, ARTHUR J. GORNEY, AND MAURICE S. SEGAL.
(Introduced by David Rapport.)

From the Department of Physiology, Tufts College Medical School, Boston.

The rôle of the parasympathetic and sympathetic nervous systems in the regulation of the blood sugar level has been studied by a number of investigators. A review of the literature impresses one first with the vast amount of work done, second with the diversified and, in many instances, ingenious methods of approach to the problem, and third with the lack of uniformity of the results obtained by the various observers.

The earlier work centered chiefly about the influence of the parasympathetic nerves over the regulation of the blood sugar level. The results in some cases pointed to the presence in the vagus nerves of secretory fibers to the pancreas, in others to the presence of tonic inhibitory fibers, and in still others to the presence of neither secretory nor inhibitory fibers. Among the methods of experimentation have been stimulation of isolated portions of the vagi and section of the same at different levels. Of the experiments in which the nerves had been cut, the majority have been performed after section either in the neck or thorax. Clark,¹ working on cats, severed the vagus nerves below the diaphragm and found increased tolerance to glucose. So far as we are aware there has been no similar work done in dogs after section of the vagus nerves at this level.

Attention has also been called to the rôle played by the sympathetic system. The methods employed here have consisted variously of lumbo-abdominal sympathectomies, splanchnic nerve sections at different levels, celiac ganglionectomies, and adrenal denervations. The effect of such procedures on tolerance to glucose and on experimentally produced diabetes has been studied and the results obtained have been just as contradictory among themselves as have been those in the case of the parasympathetic system. We have omitted reference to the large literature due to consideration of space.

*Aided by a grant from the Charlton Research Fund, Tufts College Medical School.

¹ Clark, G. A., *J. Physiol.*, 1931, **73**, 297.

Recently de Takats and his collaborators, having obtained what they regarded as significant increases in glucose tolerance in dogs after coeliac ganglionectomy, adrenal denervation, and splanchnic nerve section, have used their results as a basis for justifying splanchnic nerve section in human cases of diabetes. The results on 3 cases have been reported.² Of these, one was classified as a partial failure mainly due to incomplete operation, and another as a complete failure due to improper selection of the patient. The third case has since operation required a smaller daily dosage of insulin.

We here report the effects on glucose tolerance of the following procedures: (1) subdiaphragmatic vagus section; (2) coeliac ganglionectomy following double vagus section; (3) removal of the left adrenal and denervation of the right; (4) coeliac ganglionectomy following removal of one adrenal and denervation of the other.

The observations were made on 5 adult female dogs, weighing between 6-11 kilos each, and living on a constant maintenance diet which included cracker meal, lard, lean beef heart, bone ash, cod liver oil, and dried yeast. A fasting period of 41 hours was allowed, at the end of which time the animal was catheterized and a fasting blood sample of approximately 8 cc. withdrawn from the external jugular vein. A solution of freshly prepared 50% glucose was then injected intravenously, the amount being 1 cc. per kilo of body weight. Samples of blood were drawn at 10, 20, 40, 60, 90, 120, and in some cases 180 minutes after the time of injection. Following the withdrawal of the last blood sample, the animal was again catheterized and the urine obtained added to any that may have been voided during the course of the experiment. The blood samples were analyzed for sugar by the modified Folin-Wu method. Urinary sugar was determined by the micro Benedict method. This standardized procedure was carried out in each series of experiments performed before and after the operations.

The operative procedures were carried out with aseptic precautions and under ether anesthesia. They consisted variously of section of the vagus nerves, subdiaphragmatically, removal of one adrenal and denervation of the other, and coeliac ganglionectomy. In order to allow for recovery after operation, no observations were made until after 7-10 days, at which time healing of the wound had occurred. In each case, final observations were made several months after operation.

² de Takats, G., *Ann. Surg.*, 1935, **102**, 22.

Effect of vagus section. The effect of vagotomy on the blood sugar level curve after administration of glucose was studied in 4 dogs. In dog No. 1, after vagus section on the left side only, and in dog No. 2 after section on the right side only, there was no change in the tolerance to glucose as compared with the established normal. Following left vagus section in dog No. 2, the glucose tolerance curves showed a slight departure from the controls at points taken 40 and 60 minutes after injection. The blood sugar values obtained here were 25-30 mg. per 100 cc. of blood higher than in the controls. However, the values after 60 minutes and up to 180 minutes were in harmony with the controls for the same time interval. We do not consider the variations significant. After simultaneous bilateral vagotomy in dogs 3 and 4, the tolerance to glucose remained unchanged. In none of the observations after vagus section did the total output of urinary sugar for the period of experimentation materially change after operation.

The results found in dogs 1, 2, and 3 are shown in Tables I, II, and III.

TABLE I.
Dog No. 1.
Blood Sugar in mg. per 100 cc.

Time (min.)	Controls				After Left Vagus Section			
	Exp. 1 Wt.-9.0	Exp. 2 Wt.-9.2	Exp. 3 Wt.-8.8	Exp. 4 Wt.-8.8	Exp. 1 Wt.-8.2	Exp. 2 Wt.-8.1	Exp. 3 Wt.-8.1	Exp. 4 Wt.-8.1
0	89.3	103.1	82.0	105.3	80.6	80.1	89.7	111.1
10	215.0	204.1	250.0	235.3	206.2	172.4	168.0	206.2
20	146.0	149.2	181.8	155.0	168.0	140.8	141.8	169.5
40	121.2	126.6	136.0	139.9	124.2	102.0	113.0	103.1
60	85.5	97.6	95.2	93.9	108.1	90.9	95.2	95.7
90	87.3	94.6	87.3	91.3	92.2	88.5	94.3	98.0
120	91.3	91.3	85.1	90.9	90.5	91.7	96.1	97.1
180	—	92.6	81.3	89.3	89.3	84.4	89.7	94.3

TABLE II.
Dog No. 2.
Blood Sugar in mg. per 100 cc.

Time (min.)	Controls			After Rt. Vagus Section			After Left Vagus Section		
	Exp. 1 Wt.-10.4	Exp. 2 Wt.-10.4	Exp. 3 Wt.-10.2	Exp. 1 Wt.-10.0	Exp. 2 Wt.-10.0	Exp. 3 Wt.-10.0	Exp. 1 Wt.-9.0	Exp. 2 Wt.-8.4	Exp. 3 Wt.-6.9
0	93.0	90.1	87.3	92.6	85.1	90.9	93.0	85.8	88.5
10	215.0	241.0	238.1	204.1	186.9	243.9	—	—	198.1
20	175.4	186.9	173.9	165.3	140.0	161.3	180.2	186.0	175.4
40	104.2	102.6	93.0	117.6	—	101.5	138.9	133.3	135.1
60	79.0	80.6	71.2	93.9	84.7	81.0	124.2	98.5	117.6
90	83.3	88.5	90.5	78.7	86.2	84.0	104.2	82.3	103.1
120	87.7	100.5	86.5	72.7	90.1	90.5	94.3	77.8	105.3
180	88.5	95.7	87.7	77.8	96.6	80.6	77.5	76.3	80.0

TABLE III.
Dog No. 3.
Blood Sugar in mg. per 100 cc.

Time (min.)	Controls			After Bilateral Vagus Section			
	Exp. 1 Wt.-9.9	Exp. 2 Wt.-9.0	Exp. 3 Wt.-9.0	Exp. 1 Wt.-8.0	Exp. 2 Wt.-7.9	Exp. 3 Wt.-7.9	Exp. 4 Wt.-8.1
0	82.6	82.0	87.3	102.6	108.1	94.6	98.0
10	194.2	181.8	196.1	181.8	194.2	188.9	206.2
20	140.2	146.0	165.3	156.2	156.2	155.0	166.7
40	117.0	106.9	116.3	120.5	125.8	124.2	114.9
60	91.7	93.4	96.6	106.4	105.3	114.3	102.0
90	80.6	98.0	89.7	98.0	101.5	104.7	98.5
120	86.2	94.6	88.5	97.1	99.0	108.7	106.9
180	83.0	86.2	85.1	95.2	90.9	94.6	98.5

Effect of coeliac ganglionectomy following bilateral vagus section. In dog No. 4 in which bilateral vagus section had been performed, removal of the coeliac ganglia 13 months later had no effect on the glucose tolerance. The output of urinary sugar remained essentially unaltered. The results are shown in Table IV.

TABLE IV.
Dog No. 4.
Blood Sugar in mg. per 100 cc.

Time (min.)	Controls			After Bilateral Vagus Section			After Bilateral Coeliac Ganglionectomy		
	Exp. 1 Wt.-6.2	Exp. 2 Wt.-6.3	Exp. 3 Wt.-6.0	Exp. 1 Wt.-6.7	Exp. 2 Wt.-6.9	Exp. 3 Wt.-7.2	Exp. 1 Wt.-7.0	Exp. 2 Wt.-7.1	Exp. 3 Wt.-7.1
0	88.1	91.7	112.4	93.9	90.9	95.2	86.9	84.7	95.2
10	219.8	192.3	206.2	202.0	204.1	227.3	—	219.8	—
20	169.5	141.8	148.1	158.7	162.6	177.0	166.7	160.0	188.0
40	108.1	97.6	107.5	108.1	114.3	125.8	120.5	121.2	123.5
60	95.7	94.3	90.9	94.6	97.1	104.7	101.5	97.1	103.1
90	94.6	84.0	89.3	90.5	96.6	99.0	91.3	92.6	98.0
120	93.4	—	96.1	101.0	98.0	105.8	97.1	93.9	94.3
180	89.3	95.7	95.2	113.6	97.6	95.7	93.0	89.3	94.6

Effect of left adrenalectomy and right adrenal denervation. Following removal of one adrenal and denervation of the other in dog No. 5 the blood sugar after injection of glucose fell somewhat more rapidly than had been observed for the controls. The greatest departures from the control values occurred at the 20 and 40 minute points, the average difference being about 15 mg. for the former and 20 mg. for the latter. At points taken 60 and 90 minutes after injection, the blood sugar values averaged about 10 mg. lower than the controls. At the end of 2 hours, the values were essentially in agreement with those obtained before operation. While from this series of experiments there would appear to be some slight difference between the blood sugar curves of the

operated and the control animals, the difference, though consistent, is so small as to be of questionable significance, and insufficient for constituting a theoretical basis justifying this operative procedure as a means of increasing glucose tolerance. The output of urinary sugar was not materially altered after the operation.

The results are recorded in Table V.

Effect of coeliac ganglionectomy following left adrenalectomy and right adrenal denervation. The blood sugar values following bilateral coeliac ganglionectomy in dog No. 5 four weeks after removal of one adrenal and denervation of the other were found to agree essentially with those obtained for adrenal denervation. The output of sugar in the urine remained about the same.

The results following adrenal denervation and coeliac ganglionectomy are grouped in Table V.

TABLE V.
Dog No. 5.
Blood Sugar in mg. per 100 cc.

Time (min.)	Controls		After Left Adrenalectomy and Right Adrenal Denervation			After Bilateral Coeliac Ganglionectomy	
	Exp. 1 Wt.-6.5	Exp. 2 Wt.-6.8	Exp. 1 Wt.-6.6	Exp. 2 Wt.-6.5	Exp. 3 Wt.-6.5	Exp. 1 Wt.-6.5	Exp. 2 Wt.-6.8
0	80.0	111.1	74.3	82.3	75.2	74.3	75.7
10	202.0	198.1	—	230.0	204.1	204.1	210.5
20	160.0	152.7	146.0	151.5	131.6	111.1	137.5
40	117.6	111.1	95.2	96.1	87.7	83.7	73.5
60	93.9	93.4	79.0	88.5	79.4	85.5	82.0
90	93.9	90.9	80.0	88.9	78.1	88.1	78.1
120	97.1	86.2	87.3	88.5	82.0	80.6	85.5

The above findings confirm the results of coeliac ganglionectomy following double vagotomy in showing that removal of the sympathetic innervation has no appreciable effect on the glucose tolerance curve, although de Takats and Cuthbert³ have been inclined to call differences similar to those obtained here as significant. In this paper these authors state that "the coeliac ganglion mediates nerve impulses, the exclusion of which brings about either an increased insulin production or a reduction in the insulin requirement." We are unable to confirm the above statement. In addition, the same authors write that "in preliminary tests adrenal denervation alone has given a marked rise in tolerance." Although we have observed some changes after adrenal denervation, we are inclined to minimize their importance.

³ de Takats, G., and Cuthbert, F. P., *Am. J. Physiol.*, 1932, **102**, 614.

Summary. 1. Section of the vagus nerves below the diaphragm was found to have no effect on the tolerance of normal dogs to intravenously injected glucose. No evidence was obtained which pointed to secretory or tonic inhibitory fibers in either of the vagus nerves. 2. Removal of one adrenal and denervation of the other resulted in a slightly more rapid fall in blood sugar after intravenous injection of glucose. This difference in rate, though persistent in the animal studied, was nevertheless small and of doubtful significance. 3. Coeliac ganglionectomy after bilateral vagus section and after removal of one adrenal and denervation of the other was without effect on glucose tolerance. 4. No evidence was obtained in normal dogs to substantiate a theoretical basis for operative procedures designed to increase tolerance to glucose.

We are indebted to Dr. David Rapport for valuable suggestions in carrying out this work. We wish to express our gratitude to Drs. David D. Berlin and Joseph Tartakoff for performing all the surgical procedures.

8658 C

Calorigenic Action of Methylene Blue During Muscular Exercise.*

RUTH GUILD AND DAVID RAPPORT.

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In previous communications (Rapport,¹ Canzanelli, Segal and Rapport²) we have presented evidence indicating that while the energy of the calorigenic action of certain substances, (such as protein, certain amino acids, thyroxine, etc.) can not be used as a fuel for muscular work, that of carbohydrate and fat could be so used. The present communication deals with similar observations in regard to methylene blue, a substance foreign to the body, which, when administered, has a considerable effect on the total metabolism.

The experiments were made on a single well-trained female dog, weighing about 9 kilos, and subsisting on a constant maintenance

*Aided by a grant from the Charlton Research Fund, Tufts College Medical School.

¹ Rapport, D., *Am. J. Physiol.*, 1929, **91**, 238.

² Canzanelli, A., Segal, M., and Rapport, D., *Am. J. Physiol.*, 1934, **110**, 410.

diet, the methods being the same as those used in the above mentioned researches. The gaseous exchange was obtained in a closed system, into which, for the purpose of exercise, a horizontal treadmill was introduced. Exercise consisted of 15 minutes running at about 2.5 kilometers per hour, and the metabolism of exercise and recovery was calculated by computing the amount of respiratory exchange in excess of that at rest. This was done both on days when the resting metabolism was basal, and on days after the injection of 10 mg. of methylene blue in one percent watery solution per kilo of body weight.

Three alcohol checks were performed during the course of the work, the respiratory quotients being 0.660, 0.667 and 0.671 respectively, the maximum error in the R.Q. being 0.007, or about one percent.

TABLE I.

Date	Resting Metabolism* per hour		Increased Resting Metabolism over "A"	Work†	Excess Metabolism‡ of Exercise		Increase in Metabolism during exercise over "A"
	R.Q.	Cal.			R.Q.	Gm. cal. per kgm. of work	
Basal "A"							
1935				Kgm.			
March 27	0.92	11.8		6153	0.86	10.3	1.67
April 17	0.94	11.7		5519	0.89	10.6	1.91
" 18	0.91	11.5		5563	0.90	10.2	1.83
" 20	0.96	12.4		5457	0.94	9.9	1.82
Aver.	0.93	11.9			0.90	10.3	1.81
Methylene Blue, 10 mg. per kilo, intravenously.							
1935			%				%
May 1	0.95	16.1	35		0.96	14.5	40
" 23	0.93	17.6	48		1.10	14.6	46
Aver.	0.94	16.9	42		1.03	14.6	43

* Average of pre- and post-exercise resting metabolism.

† Running on a horizontal treadmill for 15 minutes at about 2.5 km. per hr. The term "work" is used loosely.

‡ Important to note that these excess calories are above the resting "basal" level, and not above the resting level of the same experiment.

The results are shown in Table I. In 4 experiments under "basal" conditions, the resting metabolism was 11.9 cal. per hour. When the animal was exercised, there was an "extra" metabolism which averaged 1.81 ± 0.07 gm. cal. per "horizontal" kilogrammeter of work.

After giving methylene blue, the resting metabolism averaged 16.9 cal. per hour, an increase over the basal of 42%. The extra metabolism due to exercise has been calculated as the excess above the "basal" resting level. As has been indicated in previous com-

munications, this is simply a matter of convenience, to illustrate the point made. Calculated in this way, the energy consumption during exercise in the methylene blue experiments averaged 2.59 gm. cal. per kg. of work, which is 43% higher than the exercise metabolism in the "basal" experiment. In other words there was complete summation of the metabolism of work and that of the calorogenic action of the methylene blue, showing that none of the latter could be used to provide energy for the oxidative phase of muscular exercise—another instance of specificity, that is, the failure to use certain exothermic oxidative reactions, in this process.

In the experiments of Rapport¹ it appeared that the calorogenic action of carbohydrate was abolished during exercise. In the present experiments the calorogenic action of methylene blue was, as shown by the R.Q., predominantly at the expense of the oxidation of carbohydrate, yet this "waste heat" persisted when the animal exercised. It seems to follow that the calorogenic action of the methylene blue is not due to the so-called specific dynamic action of sugar, and also that the latter is not due to "waste" necessarily accompanying its oxidation.

8659 C

Structures Developed in Amphibians by Implantation of Living Fish Organizer.

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The study of mutual interactions between developing fish and amphibian tissue was tested by implanting parts of fish blastulae into the blastocoele of developing amphibians. This method applies also to the study of the capacity for differentiation of isolated embryonic cells of the teleostean. Halves of blastulae of the eggs of *Danio rerio* (the Zebra fish), isolated from their yolk an hour before the commencement of the visible processes of gastrulation, were implanted into the blastocoele of *Triturus torosus* eggs. The hosts were fixed after 10 days of development.

The developing fish and amphibian cells are mutually compatible during gastrulation and the stages immediately following. Thus

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developing tissue of young embryos from different classes of vertebrates at first possess considerable tolerance, even though a marked tissue specificity characterizes the adults. In the present series of experiments, the implanted fish material developed wherever located: in the center of the yolk, between yolk and epidermis, or surrounded by amphibian mesenchyme. The fish mesenchyme mingled with that of the amphibian to form a composite tissue. The tremendous difference in size between the fish and the amphibian cells is an aid in distinguishing the source of the tissue.

The amphibian epidermis is always locally thickened adjacent to an implant. In some cases there were extensive epidermal proliferations, sometimes richly provided with chromatophores though formed from normally unpigmented belly ectoderm. One of these bore an extension simulating in structure an amphibian dorsal fin.

In contrast to these cases of doubtful induction, another embryo shows a true one. Fish notochord induced the formation of amphibian nervous tissue from amphibian ventral ectoderm. It is clear that the processes which normally take place in the organizer-region of the fish can stimulate amphibian cells to differentiate specifically. The fish-organizer did not induce the formation of a complete embryonic axis, but rather a single localized portion of the nervous system. The induction consisted of well-differentiated unilateral medulla, an auditory ganglion and an auditory vesicle. The induced medulla formed by the amphibian cells was similar in configuration to normal amphibian medulla; it was coextensive with the fish notochord in length. In contrast to this, the induced auditory vesicle was situated anterior to the fish notochord and the induced amphibian medulla, and probably was induced directly by the amphibian medulla.

The organizer-region of the *Danio* gastrula is only about one twenty-fifth the size of the *Triturus* organizer. The induced medulla was similar in size to the normal *Triturus* medulla and considerably larger than the medulla of the *Danio* embryo. The fish organizer has stimulated a specific reaction, the differentiation of medulla, from a mass of tissue far greater than would have been involved in comparable differentiation in the *Danio* embryo. The qualitative action of the organizer is thus clearly independent of precise size-relationships between the masses of stimulating and reacting cells.

In addition, the qualitative action of the organizer is independent of the precise age of organizing and reacting cells to a surprising degree. In the *Danio* eggs comparable in stage to the donors of

the implants, all of the processes of gastrulation and the formation of the primitive embryonic structures (somites, gut, nervous system and optic vesicles) were accomplished within the 12 hours immediately following the implantation operations. During this 12-hour period, the Triturus eggs barely completed the latter part of the processes of gastrulation. The differentiation of the induced Triturus structures was stimulated by an organizer in which the developmental processes were proceeding at a greater rate and at a more advanced period of time than the corresponding processes in the Triturus organizer. It may be inferred that the rate of reaction of the differentiating cells is inherent within those cells, whatever the rate of action of the organizing system. Further experiments are necessary to answer the problems concerned with time-relationships between organizing and responding cells.

Many of the problems formerly attacked by xenoplastic grafting can now be subjected to new analyses by interclass embryonic grafting. The demonstration of the compatibility between embryonic fish and amphibian cells and the capacity of fish-organizer to induce amphibian structures renders possible further attack upon interrelationships between the organizer and the cellular system responding to its stimulation.

8660 C

Quantitative Relation Between Follicle Stimulating and Luteinizing Effects in Castrate and Menopause Urine.

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We have reported¹ the observation that both follicle stimulating and luteinizing effects could be produced with urine obtained from castrates and women past the menopause. Numerous investigators (Zondek,² Hamburger,³ Leonard and Smith,⁴ and Col-

* Hiram N. Vineberg Research Fellow.

¹ Frank, R. T., Salmon, U. J., and Friedman, R., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **32**, 1666.

² Zondek, B., *Hormone des Ovariums und des Hypophysenvorderlappens*, 2nd Ed., Wien, J. Springer, 1935.

³ Hamburger, C., *Acta Path. et microbiol. Scandinav.*, suppl., 1933, **17**, 1.

⁴ Leonard, S. L., and Smith, P. E., *Am. J. Physiol.*, 1934, **108**, 22.

lip⁵) have described strictly follicle stimulating response to the extracts of urine of castrates, in sharp contrast to that obtained with the urine of pregnancy in which luteinization was regularly noted. On the other hand, Österreicher,⁶ Lassen and Brandstrup,⁷ Evans,⁸ and ourselves,¹ have demonstrated luteinizing effects with castrate urine extracts.

During the course of the investigation of a group of 43 cases we noted that extracts prepared from urine specimens collected from the same patient on different days produced follicle stimulation or luteinization, or combinations of both effects with equal amounts of the extracts. This led us to search for the cause or causes responsible for these variations in ovarian response. A group of 23 cases (17 surgical castrates, 3 X-ray castrates, and 3 physiological menopause) was selected from the larger group. This study is based on 76 extracts, all of which were shown to produce luteinization.

The extracts were prepared by our usual technique. In each case the equivalent of 100 cc. and 50 cc. from the same specimen of each patient was injected into test rats of 24-26 gm. in weight. All animals in these experiments were killed 96 hours after the beginning of the injections. Depending upon the effect produced by these trial quantities, the dosage of the same extract was either increased or decreased in another group of animals. With some extracts the equivalent of 100 cc. gave a luteinizing reaction, in others only follicle stimulation, and in still others a combination of both. When merely follicle stimulation resulted, other rats received increased dosage. If on the first trial luteinization was obtained the dosage was then decreased in other animals.

With some extracts very small variations in dosage produced striking differences in ovarian response. For example 100 cc., 50 cc. and 40 cc. gave III reaction; 30 cc. I in one instance; in another 108 cc. gave III and I, 100 cc. a I. In 48 titrations similar results between I and III could be obtained at will by using different quantities. The 3 photomicrographs demonstrate the extent and completeness of the reactions.

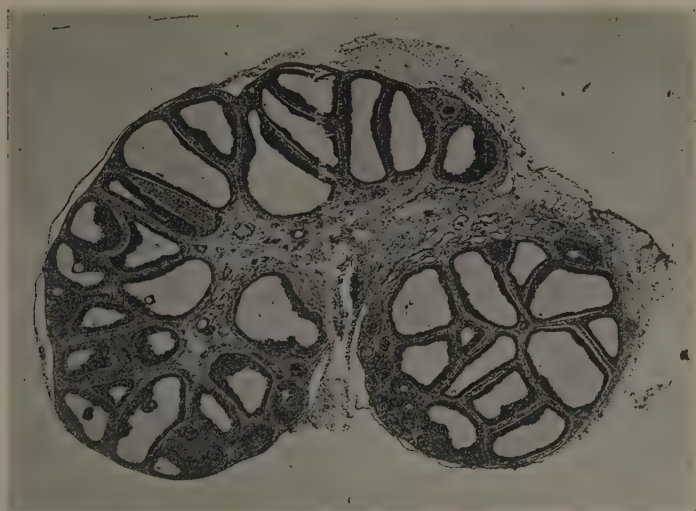
Summary: From these results it is evident that extracts pre-

⁵ Selye, H., Collip, J. B., and Thompson, D. L., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **32**, 800.

⁶ Österreicher, W., *Klin. Wochenschrift.*, 1932, **11**, 813; *ibid.*, 1935, **14**, 1570.

⁷ Lassen, H. C. A., and Brandstrup, E., *Acta obst. et gynec. Scandinav.*, 1934, **14**, 89.

⁸ Evans, H. M., and Simpson, M. E., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **32**, 1046.



FIGS. 1, 2, and 3.
Stimulation of Immature Rat Ovaries Obtained with Castrate Urine.
Fig. 1, Follicle stimulation, Reaction I.

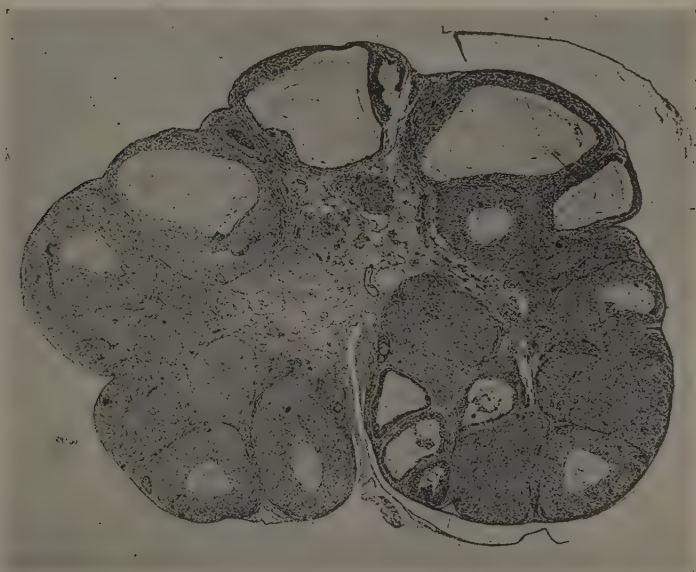


Fig. 2, Approximately equal follicle stimulation and luteinization, Reactions I and III.

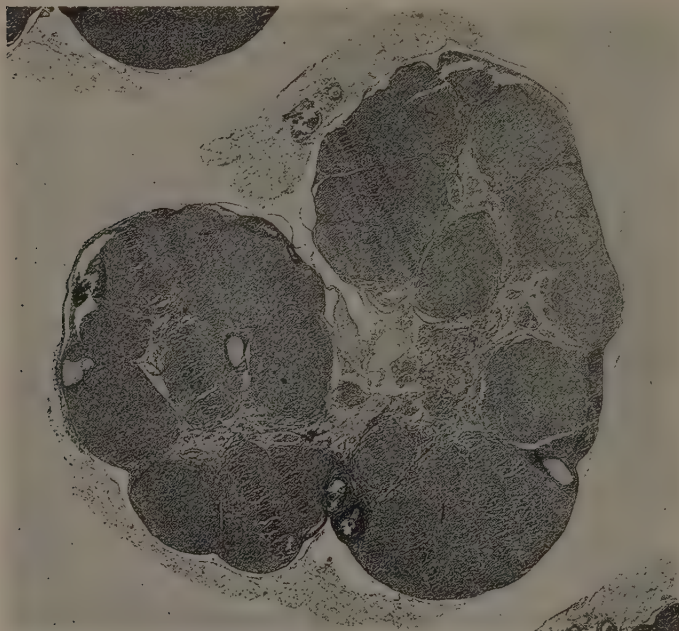


Fig. 3, Complete luteinization, Reaction III.

pared from the urine of castrates as well as those from women in the physiological menopause consistently produce both follicle stimulating and luteinizing effects. By increasing or decreasing the dosage of a given extract (in 48 specimens) we were able to produce separate effects, *i. e.*, either follicle stimulation or luteinization in immature rat ovaries. Our results do not allow us to conclude, however, whether one factor at different dosage produces both effects or whether 2 factors are present with different dosage thresholds. Further investigations to decide this fundamental question are under way.

8661 P

Bilirubinemia and Bromsulphalein Retention.

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We have previously reported¹ that reticulo-endothelial blockade produced by the intravenous injection of India ink or saccharated iron oxide leads to a retention of intravenously injected bromsulphalein. That bromsulphalein disappears from the blood in normal animals faster than it is excreted in the bile has been recognized by various workers. This observation is in harmony with the occurrence of retention after reticulo-endothelial blockade. Theoretically therefore, bromsulphalein retention may be due to either a dysfunction of the reticulo-endothelial system or a defective hepatic excretion or both. In some experiments designed to study this relationship it was considered necessary to determine whether there is any mutual interference between bilirubin and bromsulphalein with respect to their rates of elimination from the blood stream. The present report is concerned with the effect of injected bilirubin on bromsulphalein elimination.

The experiments were performed upon dogs, both normal and anesthetized. Pure bilirubin (Eastman Kodak Company) dissolved in distilled water with the aid of a few drops of 10% sodium hydroxide solution was used. Bromsulphalein tests were performed in the usual way, using 2 mg. per kilo of dye and determining the dye concentration in plasma samples obtained at 5 minutes and at 30 minutes, the reported figures for each test indicating the percentage of dye in a 5-minute sample and in a 30-minute sample respectively. Five mg. per kilo was given to 4 normal dogs. In one dog a bromsulphalein test was run immediately and showed no retention (10-0). In the other 3 the bromsulphalein was injected 30 to 180 minutes after the bilirubin. Very slight retentions occurred (20-0, 30-5, 15-0 respectively). Ten mg. per kilo was given to 3 normal dogs. Thirty to 180 minutes later the bromsulphalein tests showed retentions of 40-10, 20-0, and 40-10 respectively. In one dog the test was repeated—injecting the bromsulphalein immediately after the bilirubin. Normal figures (10-0)

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¹ Mills, M. A., and Dragstedt, C. A., *Proc. Soc. Exp. Biol. and Med.*, 1936,

were obtained. Five mg. per kilo of bilirubin produced but a slight and transient bilirubinemia, the plasma concentrations decreasing from a range of 3.3 to 4.3 mg. per 100 cc. at 5 minutes to nonrecognizable amounts in 90 minutes. After 10 mg. per kilo the plasma concentrations of bilirubin declined from a 5-minute range of 7.2 to 10 mg. per 100 cc. to nonrecognizable amounts in 120 minutes. In order to maintain a more persistent bilirubinemia 2 injections of 10 mg. each at an interval of 30 minutes were made into a normal dog. Thirty minutes after the second injection this animal showed a dye retention of 50-15. In 2 other animals 10 mg. of bilirubin was injected while they were under ether anesthesia. This definitely prolongs the bilirubinemia. These animals showed dye retentions of 60-30 and 70-50 respectively. Short periods of ether anesthesia in our experience have had no effect on the bromsulphalein test. Anesthesia of 2 to 4 hours causes a very slight retention² but has never in our experience resulted in such marked retention as this.

These experiments have been limited on account of the cost of the bilirubin, but we believe that they definitely show that an artificially induced bilirubinemia causes a retention of bromsulphalein. It has been recognized by various workers^{3, 4} that after experimental obstruction of the bile duct, there is no appreciable dye retention until a definite bilirubinemia develops and that the degree of dye retention then increases somewhat proportionately with the bilirubinemia. Rosenthal³ interpreted the failure to obtain dye retention immediately after experimental obstruction as indicating that the liver cells, if uninjured, can take up the dye, even though they cannot excrete through the biliary channels in the normal way. Our observations suggest a different interpretation, namely that the dye retention is secondary and related to the bilirubinemia. The further implication of this would be that while dye retention might occur without jaundice, marked jaundice could not occur without dye retention. As far as we know this is in accord with the reports in the clinical literature.

² Rosenthal, S. M., and Bourne, W., *J. A. M. A.*, 1928, **90**, 377.

³ Rosenthal, S. M., *J. A. M. A.*, 1924, **83**, 1049.

⁴ Snell, A. M., Greene, C. H., and Rowntree, L. G., *Arch. Int. Med.*, 1925, **36**, 273.

8662 C

Common Bile Duct Obstruction and Anaphylaxis.

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We have elsewhere reported that blockade of the reticulo-endothelial system with India ink or saccharated iron oxide does not materially modify anaphylactic shock in the dog. In the preceding paper we have shown that the hyperbilirubinemia resulting from injections of bilirubin leads to a retention of bromsulphalein. Klein and Levinson¹ and more recently Mills and Dragstedt² have reported studies indicating that reticulo-endothelial blockade causes a retention of bromsulphalein. Interpreting the dye retention from a hyperbilirubinemia as a form of physiological blockade of the reticulo-endothelial system it seemed worth while to repeat our anaphylactic studies on jaundiced animals. Theoretically at least, the blockade occurring in dogs with obstructive jaundice should be more complete than that produced with foreign materials as it is constant and progressive.

Anaphylactic shock was induced in 8 horse serum sensitized dogs in which the common bile duct had been obstructed. In one animal the obstruction preceded the sensitization, in the remainder the operation was done after the sensitization and at varying intervals before the test or assaulting injection of serum. Dye retention tests and determinations of the plasma levels of bilirubin were made just prior to the assaulting dose. For this the animals were anesthetized with ether and sodium barbital and the degree of shock evaluated by the blood pressure records as previously described.³ The essential data are shown in the accompanying table.

Severe to fatal reactions occurred in all but one animal. This animal was in poor condition and the blood pressure was extremely low before the horse serum was injected. No additional effect was produced. In 2 animals the shock was somewhat slower in developing than is usually the case. There is therefore no evidence of any mitigating effect on anaphylactic shock. Actually the per-

* Frederick Robert Zeit Fellow in Pathology.

¹ Klein, R., and Levinson, S. A., *PROC. SOC. EXP. BIOL. AND MED.*, 1933, **31**, 179.

² Mills, M. A., and Dragstedt, C. A., *PROC. SOC. EXP. BIOL. AND MED.*, 1936, **34**, 228.

³ Dragstedt, C. A., and Gebauer-Fuelnegg, E., *Am. J. Physiol.*, 1932, **102**, 512.

TABLE I.
Anaphylactic Shock in Dogs with Obstructive Jaundice.

Exp. No.	Sensitization period days	Obstructed days	Plasma Bilirubin mg. per 100 cc.	Bromsulphalein test		Degree Shock	Remarks
				5 min. %	30 min. %		
203	33	4	2.57	60	40	+++	Slow development
204	35	6	5.70	60	40	0	Bl. pr. at shock level before injection of serum
205	14	2	1.0	—	—	+++++	
206	17	5	14.4	80	60	+++++	
207	33	7	4.09	80	50	+++++	Slow development
210	17	8	14.0	60	45	++++	
212	21	12	11.7	—	—	+++++	
213	12	14	18.0	80	50	+++++	

centage of fatal reactions obtained is higher than that occurring in a large series of normal animals. In this connection it is interesting to note that Ravdin⁴ has reported that there is an increased content of histamine in the livers of dogs after common duct obstruction, in view of our general hypothesis that histamine is the causative agent of the vascular reaction and death in anaphylactic shock. If the bilirubinemia produced by common duct obstruction may be interpreted as producing a reticulo-endothelial blockade, these results then confirm our observations in blockade produced by other agents.

8663 P

Blood-CNS Barrier in Experimental Poliomyelitis.*

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A selective mechanism regulating the exchange of substances between the blood and the central nervous system, and usually referred to as the hemato-encephalic or blood-brain barrier, has long been known to exist. McIntosh and Fildes¹ showed that after intravenous injection of salvarsan no arsenic could be found in

⁴ Ravdin, I. S., *Arch. Surg.*, 1929, **18**, 2191.

* The experimental work was supported by grants from the Rockefeller Foundation to the University of Chicago.

¹ McIntosh, J., and Fildes, P., *Proc. Roy. Soc., B*, 1914, **88**, 320.

the brain, although it could be detected in tissues outside the central nervous system. Friedemann and Elkeles,² among others, believe that the mechanism controlling the passage of substances between the blood and the brain is localized in the cerebral capillaries and is intimately concerned with the permeability of the vessel walls. Trotter³ has emphasized the virtually complete isolation of nervous tissue from the somatic tissues, a point of considerable importance in the pathogenesis of neurotropic virus diseases and one frequently overlooked in procedures devised to confer immunity against these viruses. Thus, W. S. Gordon,⁴ using louping-ill virus, found the central nervous tissue of sheep difficult to immunize. F. B. Gordon,⁵ of this laboratory, attempting to immunize Rhesus monkeys to poliomyelitis virus, experienced the same difficulty.

The striking ineffectiveness of the vascular route of inoculation in producing poliomyelitis in Rhesus monkeys (*Macaca mulatta*), unless comparatively huge amounts of virus are used, presumably rests on the ability of an intact blood-CNS barrier to keep injurious substances of hematogenous origin from reaching the nervous tissues. Using the technic devised by Sawyer and Lloyd⁶ for a yellow fever protection test, we attempted to damage the barrier by intracerebral injection of sterile starch.

Nine normal and 2 immune monkeys were given intravenously a single injection of 10 cc. of a rapidly centrifuged, 10% crude virus-cord suspension. Three of the normal animals and both immune animals were injected intracerebrally with one cc. of a sterile 2% starch solution.

The 6 controls, given only virus intravenously, survived without symptoms. Of the animals receiving starch and virus, the 3 normals succumbed to infection and the 2 immunes failed to react.

It was assumed that inasmuch as poliomyelitis virus has a marked affinity for nervous tissue, it should also cause infection of the central nervous system by traveling up nerve trunks after absorption from a site of nerve injury. Large peripheral nerves (femoral, sciatic or vagus) were sectioned in 7 monkeys and 10 cc. of a 10% crude virus suspension was injected intravenously into each monkey 4 hours later. Two of the animals succumbed

² Friedemann, U., and Elkeles, A., *Lancet*, 1934, **1**, 719 and 775.

³ Trotter, W., *Brit. Med. J.*, 1926, **2**, 103.

⁴ Gordon, W. S., *Brit. Med. J.*, 1934, **1**, 885.

⁵ Gordon, F. B., Personal communication.

⁶ Sawyer, W. A., and Lloyd, W., *J. Exp. Med.*, 1931, **54**, 533

to infection, 2 showed a questionable rise in temperature and 3 failed to react to the virus. The advent of poliomyelitis after sub-infective intravenous doses of virus when nerves are sectioned adds support to the existence of an insulatory mechanism between the nervous system and the blood.

Normally well isolated from the rest of the body,⁸ nervous tissue is protected from noxious agents in the vascular system. Trauma to the nervous system, either central or peripheral, breaks down the mechanism regulating the exchange of substances between neural and somatic tissues, and permits the virus to parasitize susceptible nerve cells.

We⁷ have previously shown that sectioning the olfactory tracts prevents infection from the blood stream, even when large intravenous doses of virus are injected. Since intranasally instilled virus is highly successful in infecting monkeys and intravenous inoculation with small doses of virus unsuccessful unless neural tissue is damaged, we interpret the results of the experiments recorded at this time as further evidence that poliomyelitis virus passes directly from the nasopharynx to the brain via the olfactory tracts.

8664 C

Effect of Large Doses of Progesterone in the Female Rat.

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In a previous communication we drew attention to the fact that large doses of œstrone markedly enlarge the pituitary, the adrenals and the corpora lutea in the rat.¹ The mammary glands were also greatly developed, but this development was seen only in the normal and not in the hypophysectomized rat. More recently, our findings have been confirmed by Reece, Turner and Hill,² although

⁷ Lennette, E. H., and Hudson, N. P., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **32**, 1444.

¹ Selye, H., Collip, J. B., and Thomson, D. L., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **32**, 1377.

² Reece, R. P., Turner, C. W., and Hill, R. T., *PROC. SOC. EXP. BIOL. AND MED.*, 1936, **34**, 204.

other investigators claimed to have obtained mammary^{3, 4, 5} or at least nipple⁶ proliferation with œstrin in hypophysectomized animals. Freud and de Jongh⁷ state that the mammary effect of œstrin is greatly synergized by simultaneous administration of progesterone, both in the normal and the hypophysectomized rat. Since the corpus luteum hormone is generally looked upon as one of the most important factors in the development of the mammary gland, we were surprised to see that simultaneous administration of progesterone and œstrone to ovariectomized rats did not lead to more marked mammary proliferation than would have been elicited by œstrone alone.⁸

In connection with these earlier experiments, it seemed of interest to study whether large doses of synthetic corpus luteum hormone would have an effect upon ovaries, adrenals and pituitary similar to that previously observed with œstrone. Six normally cyclic 5-month-old female rats were injected with 4 mg. of synthetic corpus luteum hormone in corn oil subcutaneously for a period of 12 days. At autopsy on the 13th day, it was observed that their ovaries were subnormal in size, averaging 30 mg. as compared with 39 mg. in the controls. Histological examination showed the absence of any recent corpora lutea or mature follicles. The pituitaries weighed on an average 13.5 mg. as compared with 10.5 mg. in the normal. Histologically, the most outstanding change was the presence of a great number of small cells with relatively dense chromatin. The adrenals did not differ significantly either in weight or in histological appearance from the controls. The thymus was smaller in the injected animals (average 110 mg.) than in the controls (average 205 mg.). The mammary gland was quite undeveloped and differed in no way from that of a normal, untreated animal. The vagina was not cornified and not mucified, only here and there did the epithelium include small cysts filled with mucin. This explains why the vaginal smears became dioestrous within 2 days after the first injection, and remained dioestrous throughout the experiment. The endometrium was dioestrous in type, not frankly progestational.

³ Ruinen, F. H., *Acta Brev. Neerl. Physiol.*, 1932, **2**, 161.

⁴ Asdell, S. A., and Seidenstein, H. R., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **32**, 931.

⁵ Nelson, W. O., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **33**, 222.

⁶ Lyons, W. R., and Pencharz, R. I., *PROC. SOC. EXP. BIOL. AND MED.*, 1936, **33**, 589.

⁷ Freud, J., and de Jongh, S. E., *Acta Brev. Neerl. Physiol.*, 1935, **5**, 47.

⁸ Selye, H., Browne, J. S. L., and Collip, J. B., *PROC. SOC. EXP. BIOL. AND MED.*, 1936, **34**, 198.

While previous experiments on ovariectomized animals⁷ showed that the simultaneous administration of œstrin plus corpus luteum hormone causes mucification of the vagina and progestational proliferation of the endometrium, the present experiments indicate that progesterone alone has no effect on the vagina itself. The dicestrous condition of the vagina observed after progesterone is more likely to be the sequence of the ovarian atrophy caused by this hormone.

Summary. Daily administration of 4 mg. of synthetic progesterone to normally cyclic female rats causes immediate cessation of cycles, without vaginal mucification. There was ovarian atrophy, hypertrophy of the pituitary, and slight atrophy of the thymus. It has no effect whatsoever upon the condition of the mammary gland. Since œstrin is also without any direct effect upon the mammary gland (as shown in hypophysectomized animals) the effect of ovarian hormones on the mamma must be largely dependent upon the presence of pituitary secretions.

8665 C

A New Approach to the Therapeusis of Hemolytic Streptococcal Infections.

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The approach to this problem has had as its objective the *in vivo* transformation of virulent organisms into their avirulent phases as indicated previously.¹ Such objective became a possibility following our demonstration in 1915-17² (and later) that streptococci may have pleomorphic granular bacilli as at least one of their avirulent dissociants. The isolation of such strains from blood-cultures of untreated patients convalescent from streptococcal infections lent support to the above working-hypothesis.³ It suggests, perhaps, a natural tendency on the part of the host to combat the parasite by dissociating it into a less virulent, or perhaps into an innocuous form.

¹ Mellon, R. R., and Cooper, F. B., *Proc. Soc. Exp. Biol. and Med.*, 1935, **33**, 451.

² Mellon, R. R., *J. Bact.*, 1917, **2**, Nos. 2, 3, 4.

³ Koch, K. R., and Mellon, R. R., *J. Bact.*, 1930, **19**, 25.

Attempting to reinforce such tendency we have employed immune horse-sera prepared from 2 anomalous dissociants of an hemolytic streptococcus from a human case of cellulitis. Patients responding to this treatment often show hemolytic streptococci in a definite transition from their mucoid or matt to intermediate smooth, or to rough colony-forms. In several cases of marked severity this colonial change was correlated with a clinical crisis following the treatment; and sometimes the transformation appeared in all colonies isolated from several foci. At other times the *in vivo* dissociation may be initiated in large smooth colonies of hemolytic streptococci which in later sub-culture may dissociate into avirulent, non-hemolytic diphtheroids.¹ Definite antigenic community between the latter and their antecedent hemolytic streptococci may be present and will be reported by Hadley. It will be necessary, of course, that a sufficient number of cases show this dissociative phenomenon before relating it finally to the mechanism of recovery.

Summary of therapeutic results: Over a period of nearly 10 years we have treated and carefully studied 118 cases of general hemolytic streptococcal infection. They comprise 63 cases of cellulitis (including 9 of abscess) with 85.7% recovery; 28 cases of erysipelas and erysipeloid with 100% recovery; 3 of septic sore throat, with 100% recovery; 14 of acute sepsis (some complicating a cellulitis) with 71.5% recovery; 5 cases of mastoid infection taking clinically a septic course with 100% recovery; also, 2 cases of acute infectious thrombophlebitis, and 3 of lymphangitis, all of which recovered. No meningitic or puerperal infections have been treated. Of the total, 104 cases recovered and 14 died, a mortality of nearly 12%. The highest mortality occurred in the fulminating cases; that is those having the organism in the blood-stream within 48 to 72 hours.

Of special interest was the age-grouping of the erysipelatous cases, all of whom recovered; 6 were from 6 months to one year; 4 from one to 6 years; none from 6 to 12 years; 8 from 12 to 50 years. Ten were over 50, the oldest being 76. The mortality at the extremes of life is very high, particularly in infants, where it is scarcely less than 75 to 80%. Only 7 of our cases fell in the age group between 15 and 50 years, where the mortality is normally sufficiently low to bring the average down to 8 or 10%.

When it is considered that the 2 antisera employed contain no neutralizing antibodies for the toxins, either of erysipelatous or scarlatinal streptococci, some other *modus operandi* must be postu-

lated in explanation of the clinical results. Nor can we as yet assign an exclusively important rôle to bacteriotropins (opsonins), even though one of the sera appears to contain them in appreciable amounts. These are applicable to a rather wide range of strains, not being in any sense type specific. However, most of the tested cultures were not freshly isolated.

In connection with the question as to the extent to which such sera are specific, it should be realized that this is not an indispensable requisite of our approach to the problem. That is to say, bacterial dissociation may be induced quite as readily by certain environments that do not contain specific factors as by those that do. Moreover, when one considers that in streptococcal infections the mouse-protection test has not proved a reliable index of therapeutic potency—even in the mice themselves—the precise rôle of such so-called protective antibodies in the patient still awaits convincing demonstration. The purely experimental approach then, has not given us definite information on the mechanism of recovery, once infection is under way.

Yet, if the effects of this mechanism prove to be faithfully reflected in dissociation of the parasite, such effects may ultimately direct us to their causes. It is for this reason that our primary concern has been to establish a clinical phenomenon sufficiently successful to justify following its leads—whether they be in the direction of antibodies *per se*, or other tissue-functions associated with them.

That certain of these tissue-functions of the host may conceivably condition antibody-action is suggested by a rather constant fluctuation in the calcium-phosphorus product in the blood. Summarized, it appears that this product is commonly lowered during the active stages of the infection, returning to normal limits during convalescence, or thereabouts. And this, regardless of whether a complete therapeutic calcium regime is given in addition to the serum. In contrast to a normal product of 40 ($\text{Ca } 10 \times \text{P } 4$), a value of 30 or below is common and 20 has occasionally been observed. This diminution is usually decidedly more at the expense of the phosphorus than the calcium; the latter rarely falling as low as 8, while the phosphorus often reaches 2. Nothing approaching tetany-values for the calcium has ever been observed. Paradoxically, an occasional fulminating—or at least a very severe case—may have rather slight change.

The evidence as to the significance of these findings is as yet inconclusive; in fact, the approach as a whole is still regarded as

being in the experimental stage. An added uncertainty concerns itself with the instability of the strains employed in the development of the sera. And we agree with the recent conclusions of Colebrook,⁴ whose experience has made him averse to the conventional practise of submitting such sera to clinical trial generally—and as so often has proved, prematurely. But regardless of the ultimate validity of these working hypotheses we believe the clinical results to be sufficiently decisive to justify thorough inquiry into that most fundamental problem of all serious infections, namely, the tendency to invade distant tissues, instead of remaining localized.

Although diversification of duty has of necessity given a sporadic cast to these observations, the appreciable span of years covered by them makes it desirable to record them to this extent. Control-observations with the conventional streptococcal antisera, as well as frankly nonspecific ones, have been, and are being, employed as opportunity offers.

Conclusions: 1. In a series of 118 cases of general hemolytic streptococcal infections a mortality of but 12% has attended the administration of antisera prepared from 2 anomalous dissociants of a cellulitic strain of hemolytic streptococcus. 2. The objective of the treatment has been the *in vivo* transformation of the virulent types to dissociants whose virulence is diminished or absent. Clinical bacteriological evidence of this effect is submitted. 3. Associated with recovery of the patient is a return of the calcium-phosphorus product of the blood to normal. This product is usually lowered during the active stages of the disease.

8666 C

Serologic Studies with Streptococci Isolated in Cases of Myasthenia Gravis.

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Green-producing streptococci from the muscles, foci of infection, and nasopharynges of patients with myasthenia gravis were isolated by methods already described.² A carbohydrate was ex-

⁴ Colebrook, L., *Lancet*, 1935, 1, 1083.

² Rosenow, E. C., *Internat. Clin.*, 1930, 2, 29.

tracted from a number of these freshly-isolated strains by the following method:

The sedimented growth from 3 liters of dextrose-broth was treated by boiling in physiologic salt-solution for one hour and the bacteria were then removed by centrifuging. The supernatant fluid was cooled and proteins were precipitated by adding glacial acetic acid until a cloud appeared. The proteins were then removed by centrifuging and the carbohydrate was precipitated by adding 2 volumes of 95% alcohol and a small amount of sodium acetate. After a good flocculation appeared the mixture was centrifuged. The carbohydrate sediment was then dissolved in water, reprecipitated with alcohol and sodium acetate, and then redissolved in water, this procedure being carried out 5 or 6 times. The final precipitate was dried in a stream of air and dissolved in 10 cc. of physiologic salt-solution; it was then ready for use as antigen. The activity of the carbohydrate was greatest in freshly-made solutions; it decreased about one-fourth when kept at room temperature for 2 weeks.

Precipitation occurred with the carbohydrate from myasthenia gravis strains in 30% of 31 tests made with serums from 15 persons who had myasthenia gravis, in 15% of 20 tests made with serums from 11 persons having disease other than myasthenia gravis, thought to be attributable to green-producing streptococci, and in no instance in 12 tests made with the serums from 7 normal controls. Precipitation occurred with the carbohydrates from strains of green-producing streptococci isolated from persons having disease other than myasthenia gravis in 15% of 32 tests made with the serums from 15 persons who had myasthenia gravis, in 7% of 14 tests made with the serums from 11 persons having disease other than myasthenia gravis, and in no instance in 6 tests made with the serum from 6 normal controls. In these precipitation experiments the age of serums and solutions of carbohydrate were not sufficiently considered, which led to the relatively low incidence of positive reactions. The serums from 30 patients with myasthenia gravis, kept not longer than 4 months, and freshly-made solutions of the carbohydrate of streptococci isolated from patients with myasthenia gravis, gave a positive "ring" test in 60% of cases. Filtered, glycerin-salt-solution extracts of these streptococci against the same serums gave a positive test in 46.6% of cases.

The preparation of this glycerin-extract has been described previously.³ The serums of patients with severe forms of myas-

³ Rosenow, E. C., *J. Infect. Dis.*, 1929, **45**, 331.

themia gravis regularly gave precipitation: the serums of well persons never reacted nor did control serums of patients with uncomplicated diseases of known cause, other than those related to streptococci. The test was carried out in narrow tubes incubated at 45°C. in a water bath and observed frequently over a period of 2 hours. A dark background behind a bright shaded light was used in recording the test.

The serums of patients with myasthenia gravis were tested for a slowing property on streptococci in the electrical field of the Northrop-Kunitz-Mudd cataphoretic assembly. Various sets of 4 matched antigens were used, one antigen from myasthenia gravis and 3 from various other diseases. The antigens were prepared by inoculating dextrose-broth with freshly isolated cultures in dextrose-brain broth. The sediments of 16-hour dextrose-broth cultures were kept in a dense mixture of two-thirds glycerin and one-third 25% sodium chloride solution. Dilute uniform suspensions of these sediments were then made in water containing 0.05% formalin 3 or 4 days before they were to be used. The serums were then diluted 320 times in physiologic salt-solution. One cubic centimeter of the diluted serum was mixed separately with 1 cc. of each of the bacterial suspensions and was incubated for 14 minutes at 37°C. To each tube was then added 6.5 cc. of distilled water and the readings were made at once as unknowns by a technician.

Of 22 serums of patients with myasthenia gravis that were tested, all showed selective slowing action on the strains from myasthenia gravis. Each test was considered positive only when the serum of a patient with another disease against the same 4 antigens specifically slowed the antigen of that disease without slowing the antigen of myasthenia gravis. Adsorption of the serums of patients who had myasthenia gravis with different strains of streptococci showed the myasthenia gravis strains to remove selectively the slowing effect of the myasthenia gravis serums on myasthenia gravis strains.

The serums of 10 rabbits injected for 6 weeks with different formalized strains from patients with myasthenia gravis, and of 6 rabbits immunized to different strains from other diseases, showed by precipitin tests with various streptococcal carbohydrates, by agglutination and by cataphoresis of the streptococcus, the serologic specificity of the strains from patients with myasthenia gravis.

The serums of 4 horses, each immunized to a different type of green-producing streptococcus, and the serum of a normal horse,

were also studied. The serum of the horse immunized against the strains of myasthenia gravis precipitated filtered glycerin-extracts of the strains of myasthenia gravis more markedly and in greater dilution than did the other serums, and more markedly and in higher dilution than extracts of strains from other diseases. The other serums precipitated more strongly with homologous extracts.

Thus, precipitation occurred in 56% of 37 tests with the myasthenia gravis serum and myasthenia gravis extracts, in 15% of 42 tests with this serum and extracts of strains from other diseases, in 20% of 26 tests with the other serums and extracts of the myasthenia gravis strains, and in 48% of 21 tests with the other serums and extracts of strains from other diseases.

The erythematous-edematous reaction of Foshay¹ was used in the study of cases of myasthenia gravis. Several antistreptococcal equine euglobulins were standardized to a 10% solution by volume of centrifuge-packed, wet euglobulin in physiologic salt-solution. Approximately 0.03 cc. of each of these various immune-globulin fractions, along with normal horse-serum and pneumococcal immune-globulin as controls, were injected intracutaneously into patients. Of 7 patients with myasthenia gravis who were tested, all showed a predominant flare in 10 minutes to the euglobulin fractions of the serums of horses immunized to streptococci isolated from cases of myasthenia gravis. The average area of the flares to the different globulins in these cases was as follows:

Encephalitis streptococcal immune-euglobulin	4.61 cm. ²
Arthritis " "	4.11 "
Myasthenia gravis " "	10.5 "
Pneumococcal immune-euglobulin	0.85 "
Normal horse-serum	0.37 "

Normal persons gave no such flare and patients with other streptococcal diseases reacted predominantly to the globulin from the animal immunized with the strains from their type of disease. In none of the cases of myasthenia gravis did sensitiveness to normal horse-serum confuse interpretation of the reaction.

It appears that patients with myasthenia gravis harbor a streptococcus of specific serologic type, that they have antibodies in their blood specific for this organism, and that they have in their tissues antigen of this type of streptococcus, as shown by the Foshay reaction.

¹ Foshay, L., *J. Allergy*, 1935, **6**, 360.

8667 C

A Mouse Holder for Securing Tail Blood.*

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In order to obtain blood from the tails of several hundred mice for biochemical analyses,¹ it became imperative to devise a satisfactory holder for the animals. Cages made of glass tubing shaped in the general form of a Gooch crucible holder have proved their usefulness for more than two years. The animal is confined comfortably within the tube, projecting its snout into the stem by which in turn the tube is held by a clamp attached to an iron support. This form of cage is an adaptation of holders for rats made from bottles from which the bottoms have been cut, and which have been used in this laboratory over a number of years.

Unlike the cloth case of Shadle and Sharupinski² which exposes only the head of the animal, the glass holder illustrated allows the use of the unrestricted tail from which, when punctured, blood may be drawn easily and quickly into micropipettes. In terms of the blood sample taken, accurate analytical results depend upon not only the exactness of the calibration³ of the pipettes used, but also the speed with which a sample of blood may be obtained, measured, and delivered; for mouse blood clots very quickly. An efficient holding device for mice is essential to success in securing blood samples with speed and accuracy. The cage here described meets these requirements adequately and also makes assistance unnecessary.

Eight sizes of holders have been needed to accommodate mice from 40 days of age and at 40-day intervals throughout life. The stems of all the cages are 3 cm. long; the diameter of the stems of the small holders is one cm. while for the large tubes 1.5 cm. was found to be a more satisfactory width. The smallest tube, for mice of about 12 gm., has a body 4 cm. long and 2 cm. in diameter. A holder for mice weighing in the neighborhood of 35 gm. has corresponding measurements of 7 and 3.3 cm. In operation the animal instinctively tries to draw in its tail, hence, excess space,

* Aided by a grant from the Josiah Macy, Jr., Foundation.

¹ Francis, L. D., and Strong, L. C., in preparation.

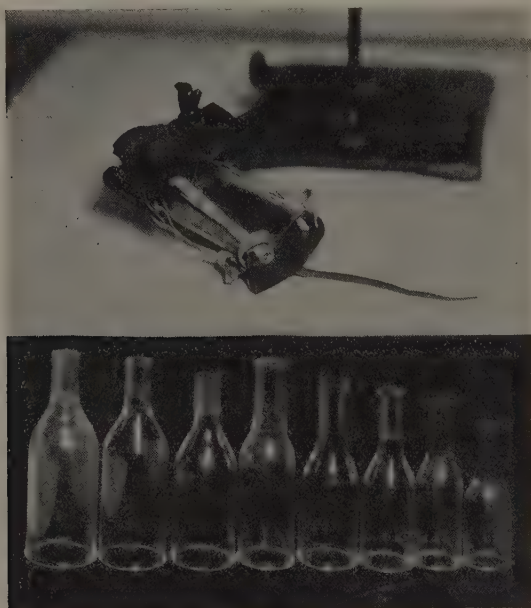
² Shadle, A. R., and Sharupinski, I., *Science*, 1935, **82**, 335.

³ Francis, L. D., *J. Lab. and Clin. Med.*, in press.

which prevents confining a mouse too closely for its well being, is better allowed in width than in length of tube.

The harness which holds a mouse in place and keeps the tail outside the cage consists of 2 squares of pliable leather fitted with metal eyelets at the corners and having a hole cut in the center of each square. One of the pieces of leather is slipped over the stem of a holder and rests on its shoulder. The other square of leather fits over the wide end; it should be larger than the width of the tube in order to prevent a mouse backing out. The center hole of this caudal square of leather, if about one cm. in diameter, gives space sufficient to avoid pressure on a mouse's tail.

Four lengths of narrow elastic are threaded through the eyelets so as to connect the 2 pieces of leather. Ends are fastened securely, one at each corner of the square to be slipped over the stem end of a holder. Each of 3 of the 4 ends attached to the caudal piece of leather is tied loosely in place.



Having in hand a holder loaded with a mouse, the harness is slipped first over the stem, the mouse's tail is then threaded through the hole designed for it, the loose end of elastic is fastened in place and the other 3 ends previously loosely tied, are adjusted

so that the caudal square of leather is held snugly against the wide end of the tube. The stem is then clamped at an angle (see illustration) which facilitates work upon the exposed tail. A mouse is thus securely but comfortably confined, hence the operator's undivided attention may be given to securing a satisfactory sample of blood.

8668 P

Arrest of Experimental Convulsions by Acetylcholine in the Cat.*

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Following a control series of experiments (15 cats) in which (1) the minimal convulsive dose of camphor monobromide,¹ (2) the total number of such dosages, (3) the total number of convulsions which could be elicited before the animal succumbed, and (4) the total amount of the drug which could be intravenously administered with 15-minute intervals between the dosages, had been established, a series of 25 cats was studied in which after establishing the minimal convulsive dose of camphor monobromide, varying dosages of acetylcholine (anhydrous solution, Chevetin-Lematte) ranging from $1:1 \times 10^{-7}$ to $1:1 \times 10^{-3}$ gm. per kilo weight were injected intravenously just before the administration of the camphor monobromide, to determine how, if at all, the convulsions would be affected.

Results. It was found: 1. That a concentration of 1×10^{-6} gm./kg. was the smallest dose of acetylcholine which would arrest the clonic convulsion following a minimal dose of camphor monobromide. 2. That while the *first* dosage at such a concentration would arrest clonic convulsions, a repetition of the same dosage after an interval of 15 to 30 minutes would only delay the onset of the clonic convulsions, for an interval of from 30 seconds to 10 minutes, and it was necessary to employ a greater concentration of acetylcholine before a complete arrest of clonic convulsions was again obtained. 3. A concentration of acetylcholine of 1×10^{-4} to

* The cost of these experiments was defrayed by a grant from the Committee on Scientific Research of the American Medical Association.

¹ Wortis, S. B., Coombs, H. C., and Pike, F. H., *Arch. Neurol. and Psychiat.*, 1931, **26**, 156.

1×10^{-8} gm./kg. would always arrest a convulsion, sometimes proving lethal. (We have found the control lethal dose of this preparation of acetylcholine in the cat ranges between 0.01 and 0.09 gm./kg.²) 4. While acetylcholine blocks the clonic element in such convulsions, it does not affect the element of tonic extension.³

From these experiments, it becomes apparent that acetylcholine has an action upon the same cells of the central nervous system as those upon which camphor monobromide acts to produce clonic convulsions.³

8669 P

Significance of Loss of Serum Protein in Therapy of Severe Burns.

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That large amounts of blood plasma are lost into burned areas has long been known; its measurement, experimentally, has shown that it may be very extensive. It is also generally realized that it is the prime, if not the only factor in the blood concentration of burned patients. But its significance in therapy has not been sufficiently emphasized.

In a series of 40 patients with severe burns, many of them fatal, the degree of blood concentration was roughly estimated by the erythrocyte count. It was found that very rapidly, often within an hour or two, the red cells increased with few exceptions to values of over 6,000,000 and in 2 cases to over 8,000,000 per cmm. Comparison of venous and capillary counts in many instances revealed significant differences only in the severe cases; in them, moreover, the counts often had to be made in venous blood because capillary blood was unobtainable. The blood was often so viscid that aspiration of a sufficient amount for chemical study even from a large vein was difficult or impossible. Nevertheless the serum protein of such blood when examined soon after admission was usually normal or low; in only one instance was

² Coombs, H. C., and Cope, O. M., *Am. J. Physiol.*, 1936, **116**, in press.

³ Coombs, H. C., and Pike, F. H., *Am. J. Physiol.*, 1931, **97**, 92.

the value above 7%, in most of them it was between 6 and 7%, in 2 it was under 6%. This in itself indicated an actual or relative loss in blood protein when one considers the marked concentration as shown by the high erythrocyte count. The degree of this loss in serum protein is apparent if we compare the average figure of about 6% in burned patients with a serum protein of 10% or more which occurs in the concentrated blood due to extreme dehydration from vomiting, diarrhea, etc. Moreover, in several patients we noted after several days of treatment still lower values for the serum protein, *i. e.*, as low as 4%. In a number of patients treated with large amounts of saline and glucose solutions we noted a falling serum protein with little or no corresponding reduction in the red cell count, thus indicating no correction of the essential concentration of the blood. The eventual return of the serum protein to normal following the usual forms of treatment was slow, requiring weeks. The albumin-globulin ratio was also determined in all patients; while 2 showed a normal value, most of them revealed a high ratio *i. e.*, between 2.9 to 3.2 indicating a loss of relatively more globulin than albumin. As the patient recovered the value returned to normal.

In many, the patient exhibited a subcutaneous edema of the unburned skin, which was especially marked when large amounts (5 to 8 liters) of parenteral fluid were given daily. In most of the serious burns there was a failure of the kidney to put out more than 10 to 20% of the amount of fluid administered.

In a number of severely burned patients intravenous acacia or blood plasma was given. Under the influence of this treatment the concentration of the erythrocytes was rapidly reduced and subcutaneous edema relieved. In several instances the red cell count dropped rapidly within 4 to 8 hours after the injection of 500 to 1000 cc. of acacia or plasma. In one patient the count fell from 8,200,000 to 6,000,000 within 4 hours after the use of 500 cc. of 6% acacia in glucose plus 500 cc. of physiological saline. Such a prompt relief of concentration was never observed following the use of saline or glucose alone.

These observations indicate that loss of serum protein is a serious result of extensive burns, and that the store of body protein is not sufficient to restore rapidly this loss when only water, glucose, and electrolyte are administered. In severe burns, therefore, large amounts of protein are needed; the injection of blood plasma is apparently more efficacious than whole blood because of the excessive concentration of red blood cells already present. In

addition to its direct effect in correction of the lost protein, it is probable that the injection of blood plasma aids the body in its resistance to infection, which is an ever-present danger in serious burns. The administration of too large amounts of water, electrolyte and glucose alone as ordinarily carried out seems not only ineffective but may, if excessive, lead to deleterious results by producing generalized edema thus lowering tissue resistance. It is possible that the process of protein replacement would be accelerated by a high protein diet particularly if beef protein and a soy bean meal were used; these proteins were found to be very rapidly converted to blood protein in experiments reported by McNaught, Scott, Woods and Whipple.¹

The serum protein was determined according to the method of Howe.²

8670 P

Intestinal Absorption of Amino Acids.

RUDOLF HÖBER AND JOSEPHINE HÖBER. (Introduced by H. C. Bazett.)

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From the investigations of Nagano¹ and of Cori² it is well known that intestinal absorption of the monosaccharides is far from being a simple process of diffusion. The different sugars introduced in equal amounts of equimolar solutions leave the intestinal cavity with very different speed, more physiological substances like glucose or galactose being absorbed much more rapidly than mannose or even the pentoses. From more recent papers of Magee and Reid,³ Wilbrandt and Laszt,⁴ Lundsgaard,⁵ Wertheimer,⁶ Verzár⁷ it can be concluded, that special cellular factors are re-

¹ McNaught, J. B., Scott, V. C., Woods, F. M., and Whipple, G. H., *J. Exp. Med.*, 1935, **63**, 277.

² Howe, Paul E., *J. Biol. Chem.*, 1921, **49**, 93.

³ Nagano, J., *Pflüger's Arch. f. d. ges. Physiol.*, 1902, **90**, 388.

⁴ Cori, C. F., *J. Biol. Chem.*, 1925, **66**, 691.

⁵ Magee, H. E., and Reid, E., *J. Physiol.*, 1931, **73**, 163.

⁶ Wilbrandt, W., and Laszt, L., *Biochem. Z.*, 1933, **259**, 398.

⁷ Lundsgaard, E., *Biochem. Z.*, 1933, **264**, 209, 229.

⁸ Wertheimer, E., *Pflüger's Arch. f. d. ges. Physiol.*, 1933, **233**, 514.

⁹ Verzár, F., *Biochem. Z.*, 1935, **276**, 17.

sponsible for the higher rates of absorption. This paper reports experiments leading to the conclusion that there exists still another group of substances of great physiological importance, the amino acids, which likewise leave the intestine with greater velocity than could be expected from simple diffusion.

The experiments were carried out with starved rats under nembutal anesthesia. In each experiment, a loop of the small intestine was isolated by ligatures and filled successively with equal volumes of equimolar solutions of a number of indifferent lipid-insoluble organic substances. After identical intervals the loop was emptied and rinsed with Ringer. After precipitation with tungstic acid the organic residue was determined, mostly by the Kjeldahl method, since the majority of the experiments dealt with amino acids as compared with amides.

If membrane diffusion is the only factor in intestinal absorption, the absorption rates of substances of the same molecular volume (m.v.) would be the same, while those of substances of different m.v. would decrease in the order of rising volumes. An illustration of the latter is found in the following experiment, in which 3 amides: acetamide (69), lactamide (98) and succinamide (126) were compared. The numbers in brackets are the m.v. calculated by applying the formula of Kopp. The percentages of absorption were found to be: 69, 30 and 5.

In experiments concerned with the comparison of amino acids and other organic compounds it must be taken into account that the diffusion coefficients of the amino acids fail to correspond to the calculated m.v., the diffusion rate being definitely smaller than expected. The reason is that the "Zwitterions" of the amino acids are strong dipoles, which, by exerting a vigorous pull on the surrounding water dipoles, form a more or less stable shell of water around the molecules. From diffusion experiments with collodion membranes carried out at our suggestion by Schmengler⁸ it can be concluded, that, for instance, glycine (76.5) penetrates the collodion membrane nearly as slowly as malonamide (104.4), alanine (98.5) as slowly as xylose (153.4), asparagine (134.2) much more slowly than xylose (153.4), leucine (164.5) slower than mannitol (189.2). If the amino acids behave in a similar manner in absorption, alanine ought to pass the intestinal wall much more slowly than malonamide. But such is not the case, since, for example, the percentage absorption of alanine (98.5) is 59.1, of malonamide (104.4) 25.6. Still more striking is the fact that as-

⁸ Schmengler, F. E., *Pflüger's Arch. f. d. ges. Physiol.*, 1933, **232**, 591.

paragine (134.2), in spite of a higher calculated m.v., penetrates the intestinal wall far more rapidly than malonamide (104.4), the percentages of absorption being 61.6 and 16.8 respectively. In another experiment, in which succinamide (126.4), asparagine (134.2) and xylose (153.4) were alternately placed in the same loop, the percentages of absorption were found to be 9.2, 46.2 and 7.7. Finally, when a mixture of malonamide plus xylose is compared with asparagine plus xylose, the following percentages of absorption are observed: malonamide plus xylose 11.7 and 13.3 respectively, asparagine plus xylose 65.6 and 10.7 respectively.

These results as well as others suggest that the amino acids, like some hexoses, exhibit preferential intestinal absorption. It may be that, in both cases, specific cellular mechanisms favoring the entrance of particularly useful substances into the body are involved. This also seems to be true of the reabsorption of substances by the kidney tubules, which, at least in the case of the frog, have been shown to retain selectively Cl ions, some hexoses (chiefly glucose and galactose) and amino acids.

The next step in our investigation will be directed towards the elucidation of the mechanism effective in the selective absorption.

8671 P

A New Rapid Method for Direct Typing of Pneumococcus in Sputum of Pneumonia Cases.

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The diagnostic laboratory is not lacking in reliable methods for the typing of pneumococcal pneumonias.^{1, 2, 3} Most noteworthy, perhaps, is the Neufeld method⁴ which affords direct typing of pneumococci in the sputum by a "Quellung" or swelling of the bacterial capsule in the presence of the type-specific antiserum. In this preliminary report we shall briefly outline the technique and the merits of a new method.

¹ Avery, O. T., *J. Am. Med. Assn.*, 1918, **70**, 17.

² Kohn, L. A., *J. Am. Med. Assn.*, 1925, **84**, 1733.

³ Rosenthal, L., and Sternberg, B., *J. Lab. and Clin. Med.*, 1929, **15**, 78.

⁴ Cooper, G. M., and Walter, A. W., *Am. J. Pub. Health*, 1935, **25**, 469.

Technique: The method involves a serological test for precipitinogen in the sputum. Freshness of the sputum sample is not obligatory. A few drops of sputum suffice for the test; $\frac{1}{2}$ to 1 cc. is optimal for reasons of convenience. The sputum is treated with an equal volume of a 10% solution of saponin (Merck) in 0.9% saline. The mixture is then shaken and incubated at 37°C. for one-half hour. To aid in clarification approximately $\frac{1}{4}$ volume of a stock suspension of aluminum hydroxide* is added to the mixture with brief stirring. This is then promptly centrifuged at high speed for 15-20 minutes. The supernatant, which is clear, or at worst homogeneously opalescent, is then tested for type-specific precipitinogen by the routine "ring-test" method.

For type-specific antibody, horse antipneumococcus serum has been found satisfactory. It is important that the antiserum be clear. The undiluted type-specific antiserum is pipetted into narrow-bore precipitin tubes (2.5-3 mm. inside diameter) with a capillary pipette and is carefully overlaid with about $\frac{1}{2}$ volume of the sputum-antigen. In the homologous type antiserum, ring formation at the interface often begins to appear immediately, or after a few minutes. The tubes are incubated at 37°C. and re-observed after one-half and one hour. With the technique described above, a specific ring-test was always confirmed by the later formation of specific precipitate upon mixing antigen and antiserum in the precipitin tube. For this purpose the tubes may be re-incubated for one hour after shaking and then refrigerated over night.

It is advisable to set up a control ring-test with saponin solution in place of antigen (saponin-treated sputum). If the antiserum is turbid, non-specific rings are occasionally obtained with saponin in 1:20 dilution. This precipitation at the interface is hazy and somewhat diffuse and is readily differentiated from specific "rings." Furthermore, such precipitated material in the control tube disappears on shaking, and in no case does a precipitate reappear on prolonged incubation. These control rings have been encountered in only 3 of 54 tests. With clear antisera, this occasional complication is entirely absent; such sera never give false "rings."

Sputum samples from 22 unselected cases of lobar pneumonia

* The $\text{Al}(\text{OH})_3$ is prepared as follows: to a 10% solution of potassium alum an excess of dilute NH_4OH is added drop by drop with constant stirring until the resultant milky suspension is distinctly alkaline to litmus. The precipitate is then washed several times with water until neutral to litmus, and is then resuspended in water to about the volume of the original alum solution. This stock suspension is stable for the present purpose; however, it must be shaken before use.

have been tested by this new method. "With the first 8 samples, the $\text{Al}(\text{OH})_3$ treatment was omitted. Correct typings by means of specific "rings" were obtained within one-half hour in 20 of the 22 cases. Of the 20 successful typings, only 3 failed to give a specific precipitate after further incubation. All these 3 were sputums which had not been treated with $\text{Al}(\text{OH})_3$. All typings were confirmed by the mouse method of Sabin.⁵† The positive results include cases of *Pneumococcus* Types I, II, III, V, VI, VII, VIII, IX, XVIII, XXII. Two sputums failed to give a typing by this method. One of these, an atypical case of Type III lobar pneumonia, showed so few pneumococci in the sputum that the failure to detect specific precipitinogen was readily understandable. This sputum failed to type by the Neufeld as well as by the mouse method; the pneumococcus could be typed only after isolation in pure culture. The other sputum which failed to give a typing was a Type VII lobar pneumonia. This sputum was obtained after crisis, on the sixth day of the disease, but contained a "moderate" number of pneumococci.

Perhaps the chief objection to all other methods for detecting precipitinogen in the sputum lies in the difficulty of obtaining an antigen of sufficient clarity for a precipitin test. In the method described here, this difficulty seems to have been successfully overcome by the use of aluminum hydroxide as a clarifying agent. It may be pointed out, furthermore, that the authors believe the new test is less subjective, more easily mastered, and not significantly more time consuming than the Neufeld test. The ultimate proof of the value of this test must await a more extended series of cases.

Summary: A new rapid method for the direct typing of pneumococci in sputum is outlined. In this method, saponin is used to dissolve the bacteria⁶ and liberate the type-specific antigen. Aluminum hydroxide was employed for clarifying the sputum.

⁵ Sabin, A. B., *J. Infect. Dis.*, 1930, **46**, 469.

† For the results of the mouse typings we are indebted to the personnel of the bacteriology diagnostic laboratory of this institution.

⁶ Klein, S. J., *J. Bact.*, 1935, **30**, 43.

Diffraction Spectra of Striated Muscle and the Mechanism of Contraction.

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Previous work^{1, 2, 3} on the diffraction patterns produced by striated muscle at normal, stretched, and contracted lengths, has shown in general that s , the sarcomere length, can be calculated from measurements of the angles of diffraction, Θ_n , by means of the diffraction grating equation $n\lambda = s \sin \Theta_n$, where n = the spectral order, and λ = the wave length of the light. In the present work records have been obtained of the variations of the diffraction pattern, along with the simultaneously produced tension response, of the frog sartorius during isometric twitch. A set of only about 400 striations along the length of the muscle near the nerve plexus is used to form the diffraction spectra. It is thus possible, using the above equation, to compare changes in the length of the sarcomeres of these striations, with changes in the state of the whole muscle at each instant of muscle activity.

Fig. 1 shows 2 records obtained from different sartorii stimulated with supermaximal break shocks. Starting with the lowest band



FIG. 1.

Tension and Diffraction Variations of Frog Sartorius During Isometric Twitch.

¹ Ranvier, L., *Arch. de Physiol.*, 1874, **6**, 774.

² Vlès, F., *Propriétés Optique des Muscles* (Chapter 2), Paris, 1911.

³ Sandow, A., *Bull. Am. Phys. Soc.*, 1933, **8**, No. 5.

each record includes: the undiffracted beam, the first order spectrum, and the tension record, the dashes indicating 0.01 sec. intervals. The arrows mark the instant at which the tension response begins.*

Although the unsharp edges of the spectra permit only a rough analysis, several features are nevertheless quite plain. The first order spectra persist during contraction; hence it follows that the striations likewise persist. The upward displacement of the spectra is the consequence of an increase in the angle of diffraction; since n and λ are constant, s must decrease. The rough measurements show that at the peak of contraction the percentage decrease in s is of the same order as that of the length of the entire muscle.

The persistence of the spectra and the extent of their displacement during contraction bear on the view of Jordan⁴ that "contraction is a matter of the equal division of the dark stainable materials of the Q discs and the movement of the resultant semi-discs in opposite direction against the limiting telophragmata of a sarcomere. Relaxation is the reversal of this process." If this description is correct for isometric contraction, there should be doubling of the number of striations per unit length of muscle during the contraction period and again during the relaxation period. In our records such behavior would cause during these periods a displacement of the first order spectra to a position at approximately twice the distance of the resting first order spectra from the undeviated beam. Since this does not occur, we must conclude that Jordan's description does not hold for the isometric twitch of the frog sartorius.

Finally, it can be noticed that displacement of the spectra begins simultaneously with, if not slightly earlier than, the instant at which the tension response starts. The entire variation of the spectra during the twitch is over in about 60σ , whereas the tension record requires more nearly 90 - 100σ for completion. This means that under the conditions of our experiments the changes in the length of the sarcomeres do not parallel the changes in length of the entire muscle during an isometric twitch. A similar time difference between double refraction, and tension changes during isometric twitch has been found by von Muralt;⁵ and Gasser and Hill⁶ have

* The two arrows are not in line, since for technical reasons the diffraction, and the tension recording beams did not pass through the slit of the recording camera at the same angle.

⁴ Jordan, H. E., *Physiol. Rev.*, 1933, **13**, 301.

⁵ Von Muralt, A., *Pflüg. Arch.*, 1932, **230**, 299.

⁶ Gasser, H. S., and Hill, A. V., *Proc. Roy. Soc. B.*, 1924, **96**, 393.

concluded that the fundamental mechanical response of muscle has a different time course than that of tension development of the whole muscle. It is hoped that further research on sarcomere behavior by means of the muscle diffraction patterns will help elucidate some of these problems of the mechanism of contraction.

8673 P

Ascorbic Acid Oxidase in Determining Vitamin C in Lens and Aqueous Humor.

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The vitamin C content of the eye has been the subject of a great deal of speculation by investigators. In the normal lens and aqueous, there is an appreciable quantity of the vitamin. In the cataractous lens, however, this quantity is markedly decreased.^{1, 2}

At first, biological methods were used for determination of vitamin C. Later, it was estimated by titration with Tillman's reagent, 2, 6-dichlorophenolindophenol. It was accepted that reduction of the dye at pH 2 was entirely due to vitamin C. However, so far as the lens is concerned, this has been challenged by Fujita, Iwatake, and Miyata,³ who, by use of Folin's tungstate reagent, have found that only 79% of the reduction of indophenol depends upon vitamin C. Tauber, Kleiner, and Mishkind⁴ recently found in the Hubbard squash an oxidase enzyme which is specific for vitamin C. Use of this, then, should furnish direct evidence as to whether the substance in the lens and aqueous, which reduces the dye, is totally vitamin C.

We first undertook to substantiate the work of Tauber, *et al.* The results of our experiments on the action of the oxidase at various pH values are given in Table I.

As seen from our data, the optimum pH range of the enzyme is 5.5-6.0,—which confirms the work of the previous investigators.

¹ Mueller, H. K., and Buschke, W., *Arch. f. Augenheilkund*, 1934, **108**, 592.

² Bellows, J., *Arch. of Ophth.*, in press.

³ Fujita, A., Iwatake, D., and Miyata, T., *Biochem. Z.*, 1935, **277**, 296.

⁴ Tauber, H., Kleiner, I. S., and Mishkind, D., *J. Biol. Chem.*, 1935, **110**, 211.

TABLE I.
Effect of Reaction of Medium upon Activity of Ascorbic Acid Oxidase (Squash).

pH	Amount of Vitamin C added mg.	Amount of Vitamin C after Incubation at 37° for 30 min.	
		With Buffer alone mg.	With Buffer and enzyme mg.
4.7	.083	.034	.008
5.0	.083	.023	.005
5.3	.083	.020	.004
5.5	.083	.016	.003
5.8	.083	.011	.000
6.0	.083	.007	.000

To determine vitamin C in the lens, it was extracted twice with 5% metaphosphoric acid to precipitate the proteins, centrifuged, washed, and made to a total volume of 50 cc. A one cc. aliquot of this was titrated with the dye. Another one cc. aliquot was incubated for 30 minutes at pH 5.5 with one cc. of the enzyme extract. The solution was then brought to pH 2 with sulphuric acid and titrated. Control portions were incubated without the enzyme. Similar experiments were done upon the aqueous. The data are given in Table II.

TABLE II.
Determination of Ascorbic Acid Content of Lens and Aqueous Humor of Cattle.
1 cc. dye = 0.043 mg. Vitamin C.
Wt. lens = 4.14 gm.

	cc. of Dye	Amount of Vitamin C (Cattle Lens) mg./gm.
1 cc. lens extract	0.645	0.335
1 cc. lens extract with buffer alone (incubation)	0.620	
1 cc. lens extract with buffer and enzyme (incubation)	0.000	
		Amount of Vitamin C (Aqueous Humor Cattle) mg./cc.
1 cc. aqueous	4.650	0.199
1 cc. aqueous with enzyme and buffer (incubation)	0.000	

The results clearly indicate that at pH 2 the total reducing action of the lens and aqueous on Tillman's reagent is due to vitamin C. The fact that there was very little oxidation of vitamin C in the buffered solution without enzyme was to be expected, for, as was shown in a previous report by one of us,² the lens contains an anti-oxidative mechanism. This mechanism, however, does not, evidently, protect the vitamin against oxidation by the enzyme.

These experiments are offered as direct evidence that reduction of Tillman's reagent at pH 2 by lens and aqueous is entirely due to their vitamin C content.

8674 C

Motility of the Stomach of the Mammalian Fœtus.

M. H. F. FRIEDMAN. (Introduced by B. P. Babkin.)

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Very little is known about the reaction of the stomach of the mammalian fœtus to drugs. According to Tani¹ the stomach of the newborn rabbit is less sensitive to sympatho- and parasympathomimetic drugs than is the stomach of the fœtus. Adrenaline accelerates the spontaneous gastric movements of the rabbit fœtus whilst it has a restraining action on those of the human fœtus. Inada² observed that intestinal strips from the human fœtus were excited by parasympathomimetic and inhibited by sympathomimetic drugs. The experiments of Koshtoyantz and Mitropolitanskaya³ on the effects of adrenaline and pilocarpine on the movements of the gastrointestinal tract of the human fœtus are few and inconclusive.

The results herein recorded were obtained in experiments on the isolated stomach of the foetal cat, the newborn cat and the newborn dog. The newborn dogs were taken from the mother about 10 hours after birth. Their digestive tract was without visible signs of milk, so it is possible that the puppies had not suckled. Of the 4 newborn kittens, all less than 4 hours old, 2 had suckled and 2 had not.

The foetal cats were obtained by anæsthetizing the mother with ether and performing a laparotomy. It was found that rapidity was essential when removing the fœtuses, since handling of the uteri initiated gasping reflexes in the fœtuses causing them to swallow amniotic fluid.

A strip of stomach tissue or (where the stomach was very small) the whole stomach was attached to a lever, and suspended in a bath of mammalian Ringer solution at 37°C. The bath was of 500 cc. capacity. Air was bubbled through the solution at a constant rate with a force insufficient to disturb the otherwise sensitive recording lever.

The drugs employed were acetylcholine bromide (Eastman Ko-

¹ Tani, K., *Jap. J. Obst. Gynecol.*, 1927, **10**, 2.

² Inada, H., *Fukuoka Acta Med.*, 1928, **21**, 596 (German summary).

³ Koshtoyantz, C. S., and Mitropolitanskaya, R. L., *Fiziol. Z.*, 1934, **17**, 1309 (Russian).

dak), adrenaline hydrochloride (Parké-Davis), atropine sulphate (Merck), ergotamine metansulphonate (Sandoz), and pilocarpine hydrochloride (Merck).

Body length was measured as the distance from the crown (region of ears) to the base of the tail.

We do not know exactly at what stage in the development of the cat foetus the stomach begins to show spontaneous movements. According to Tani¹ the stomach of the rabbit foetus shows spontaneous movements when the body weight has reached 7.5 gm. Koshtoyantz and Mitropolitanskaya² claim the foetal human gastrointestinal tract is without automaticity before the 6th to 7th week. The small size of the stomach made kymographic recording difficult in cat foetuses less than 70 mm. long. According to Latimer⁴ the length of the entire digestive tube in a foetal cat 70 mm. in body length (tip of nose to anus) is only 120 mm. In a few experiments we observed movements of the stomach of cat foetuses of about 58 to 60 mm. body length but the influence of drugs on the movements could not be studied. Shaklee, Christensen and Oppenheimer⁵ noted that the iris of the foetal pig did not react to choline esters before the 71st day of gestation.

The stomach of the cat foetus as well as that of the newborn cat and dog is very sensitive to the slight succussion produced by the air bubbling through the bath. Shutting off of the air supply greatly diminishes the rhythmic contractions and also produces some fall of the tonus. Similar observations on strips of stomach from the adult cat and dog were made by Brown and M'Swiney.⁶

The contractions occurring in the foetal cat's isolated whole stomach under the conditions of the experiment take the form of either peristaltic or antiperistaltic waves. The normal peristaltic type was observed to be the more frequent. Thus in the stomach of a foetus 92 mm. body length a wave of constriction originating at or near the gastro-oesophageal junction would reach the pyloric region in 18 to 25 seconds. A second wave might commence when the first was half-way down the stomach, but usually the second wave would originate only when the preceding wave was well within the region of the pyloric antrum. Both the peristaltic and antiperistaltic waves occurred in groups, 3 or 4 constrictions following consecutively with relatively long intervals between the wave-

¹ Latimer, H. B., *Anat. Rec.*, 1934, **60**, 23.

² Shaklee, A. O., Christensen, K., and Oppenheimer, H. E., *Proc. Soc. Exp. Biol. and Med.*, 1936, **34**, 225.

⁶ Brown, G. L., and M'Swiney, B. A., *Quart. J. Exp. Physiol.*, 1926, **16**, 9.

groups. A series of 3 waves of a total duration of about 45 seconds might be followed by a resting phase of about 60 seconds or more before a new series of waves commence.

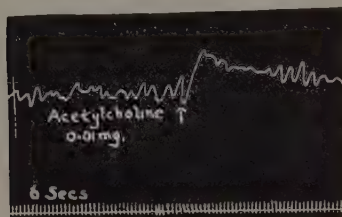


Fig. 1

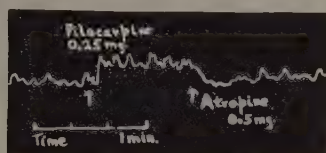


Fig. 2

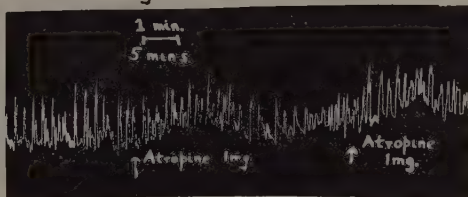


Fig. 3

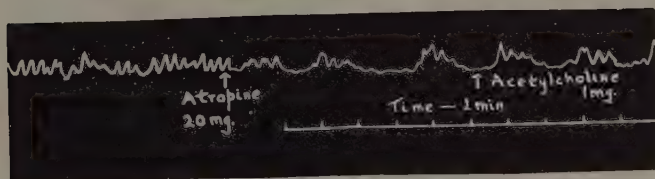


Fig. 4

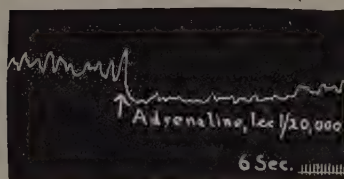


Fig. 5

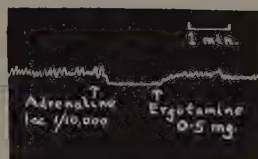


Fig. 6

All records are from the stomach of the foetal cat. In each case the capacity of the bath was 500 cc. The body lengths (crown-rump) of the foetuses were:

Fig. 1—162 mm. Effect of acetylcholine (1/50,000,000).

Fig. 2—89 mm. Atropine (1/1,000,000) abolishes the effect of pilocarpine (1/2,000,000).

Fig. 3—110 mm. Temporary rise of tonus induced by small doses of atropine (1/5,000,000).

Fig. 4—98 mm. Periodic changes in tonus and rhythmicity induced by large doses of atropine (1/25,000).

Fig. 5—162 mm. Effect of adrenaline (1/10,000,000).

Fig. 6—116 mm. Ergotamine (1/1,000,000) antagonizes the effect of adrenaline (1/5,000,000).

Parasympathomimetic drugs have a definite stimulatory effect, raising the tonus and in some instances increasing the rate of contractions of the stomach of both the foetal and newborn cat and dog. (Fig. 1). Acetylcholine initiated rhythmic movements at a higher level of tonus in those cases in which the foetal stomach did not show spontaneous contractions. The stomach in a cat foetus 98 mm. long was sensitive to acetylcholine in dilutions even in excess of 1/50,000,000, and to pilocarpine in a dilution of 1/2,500,000. The foetal stomach is slightly more sensitive to acetylcholine than is the newborn cat's stomach as shown by our experiments and also more sensitive than is the adult cat's stomach.

Atropine when given after either acetylcholine or pilocarpine antagonized these (Fig. 2) and brought about partial to complete cessation of motility. Very small doses of atropine (1/500,000) when not preceded by previous treatment of the stomach by other drugs raised the tonus for a short period of time (Fig. 3). Larger doses of atropine (1/25,000) arrested the motility of the stomach often at a high tonus level or else induced periodic tonic and rhythmic contractions which were not affected by subsequent addition of acetylcholine (Fig. 4). This twofold effect of atropine is in agreement with Magnus' theory⁷ of atropine action on the myenteric plexus.

Adrenaline had predominantly an inhibitory effect on both tonus and contractions (Fig. 5) though in low concentrations its effect on gastric tonus was frequently excitatory especially in the case of the large foetuses and the newborn cat. An initial rise in tonus was sometimes seen in stomachs from the larger foetuses; this would perhaps indicate that the stomach of the smaller foetuses were the most sensitive to adrenaline.

When acetylcholine was followed by adrenaline the reaction was sometimes synergistic but most often the result was one of antagonism. The type of response to adrenaline after acetylcholine apparently depended on the concentration of adrenaline. The occasional synergistic effect produced on the foetal stomach by these 2 drugs in combination is strikingly similar to that observed on strips of stomach of *Necturus* (Friedman⁸). Apparently no such synergism occurs in the adult cat's stomach.

Ergotamine produced only an occasional rise in tonus. However, this drug had a very marked inhibitory effect on adrenaline.

⁷ Magnus, R., *Münch. med. Wochenschr.*, 1925, S. 249.

⁸ Friedman, M. H. F., *Trans. Roy. Soc. Canada*, 1935, **29**, Sect. V, 175.

Used after adrenaline, ergotamine restored the tonus level of the muscle which adrenaline had lowered (Fig. 6).

Conclusion. Spontaneous contractions of the stomach of the cat foetus are already present in foetuses of 58 mm. body length. Both peristaltic and antiperistaltic movements can be observed. Acetylcholine and pilocarpine raises the tonus and in some instances increases the rate of contractions. The effects of these drugs are abolished by atropine. Atropine by itself has a double—inhibitory and excitatory—effect. Adrenaline in small doses stimulates and in large doses inhibits the motility. Ergotamine paralyzes the effect of adrenaline. The usual effect of adrenaline after acetylcholine is one of antagonism but sometimes the 2 drugs act synergistically.

8675 C

Tonsillitis in Dogs Due to Hemolytic Streptococci.

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Recent observations in our laboratories were reported indicating the constant presence of hemolytic streptococci of the beta type in the tonsils of the cow, sheep and hog.¹ As in man these organisms constitute part of the normal flora of the lymphoid tissue of the oropharynx of cattle, differing in certain biologic properties from the human streptococci. The rôle of these bacteria in the production of disease in animals is still undetermined. One of us² has observed the occurrence of tonsillitis in dogs with manifestations of enlarged, red tonsils, often with exudate and fever. A study was undertaken to determine the relation of the hemolytic streptococci to this tonsillar infection and the incidence of similar streptococci in normal tonsils of dogs. All of the observations were made during the months of February, March and April, 1936.

Swabs of the tonsils during the acute stage of illness in the dogs and the tonsils removed surgically from the affected dogs were

¹ Pilot, I., Buck, C., and Davis, D. J., *PROC. SOC. EXP. BIOL. AND MED.*, 1936, **34**, 233.

² Eastman, D. A., *North Am. Veterinarian*, 1936, **17**, 37.

sent in the mail by one of us (Eastman) and cultures made within 48 hours after taken. The tonsils were incised beneath the mucous membrane transversely and cultures obtained from the narrow exposed crypts. Dilution-pour-plates were made of the swabs and tonsils in infusion blood (5%) agar with 20% ascites fluid, and examinations made after 24 and 48 hours at 37.5°C.

Cultures of 14 pairs of tonsils yielded beta hemolytic streptococci in predominant number in 9 instances, in pure culture in 3. Of the 5 negative, 3 were overgrown with numerous colonies of the colon group. Three of the 4 swabs submitted yielded similar hemolytic streptococci, in one instance in pure culture. These results indicate a high incidence of beta hemolytic streptococci, often in predominant or pure numbers in cultures from acutely inflamed tonsils.

To determine the incidence of beta hemolytic streptococci in normal dogs, swabs of each tonsil were carefully obtained, avoiding contamination from mouth secretions and cultures made by the same technic. Twelve of 13 young dogs, apparently well, about 6 to 12 months of age, gave positive results for hemolytic streptococci of the beta type. In most instances the colonies numbered from 10% to 20% of the total number of colonies. In biologic properties these streptococci resembled the strains isolated from the dogs with tonsillitis.

One of these dogs developed symptoms like distemper and died of pulmonary complications. At autopsy the tonsils were not enlarged and the lungs revealed a disseminated broncho-pneumonia. Cultures from the lung and the tonsils yielded large numbers of hemolytic streptococci. These findings indicated the possible important rôle the streptococci of the tonsils may play as secondary invaders during distemper and other diseases, similar to the hemolytic streptococci of human tonsils, in the course of measles, influenza, etc.³

The young dogs mentioned above were employed in experiments to produce sore throat or tonsillitis by swabbing the surfaces of the tonsils with cultures of strains of hemolytic streptococci. Cultures of the tonsils, temperature readings, and leucocyte counts were made prior to inoculation. In the first group of 6 dogs, swabbing with broth culture (18-hour broth) of hemolytic streptococci obtained in pure culture from an acute tonsillitis in dog failed to produce any change. This strain, however, was on artificial media for several weeks, and may have lost its virulence. A

³ Pilot, I., and Davis, D. J., *J. Inf. Dis.*, 1919, **24**, 386.

second group of 7 dogs of various breeds was inoculated by swabbing the heavy suspension of organisms obtained from 18-hour ascites-broth culture of the fresh strain isolated from lungs of the dog who died from pneumonia.

Two of the 7 dogs developed a severe reaction in the throat 48 hours later. In one, the temperature rose to 104°F. on the fourth day and examination revealed enlarged red tonsils with follicular exudate and marked congestion of the vessels of the throat. The dog appeared sick and nonresponsive. Cultures yielded predominant numbers of the streptococci. The redness and congestion declined on the fifth day and the tissues and temperature became restored to normal on the ninth day. The second dog fell sick on the second day and on the fourth was very toxic, with fever of 104.4°F., marked redness and swelling of the tonsils. Death occurred on the fifth day and at autopsy the tonsils were enlarged, covered with diffuse layer of fibrinopurulent exudate. The lymphoid tissue of the pharynx was markedly engorged and the cervical lymph glands enlarged and soft. The lungs revealed an early bronchopneumonia. Cultures of the tonsils, pharynx and lungs revealed predominant numbers of the hemolytic streptococci. The other 5 dogs at times showed some redness but no exudate, elevation of temperature or marked illness. Three had nasal discharge which yielded hemolytic streptococci.

Five dogs were inoculated with a fresh strain of an encapsulated hemolytic streptococci (*S. epidemicus*) of human origin from a case of acute tonsillitis. The results were uniformly negative and at no time were the encapsulated mucoid streptococci recovered from the throat of the dogs.

This same group of 5 dogs was inoculated in a similar way with a strain isolated from the lung of a second dog who died of distemper. Two became ill and revealed enlarged red tonsils, one with exudate, yielding large numbers of streptococci. Their temperature rose to 103.2°F. and 103.4°F. Recovery was apparent on the eighth day.

Twenty-two canine strains, 9 from excised tonsils, 12 from the throat of normal dogs, and one from lung of bronchopneumonia were studied and found to conform to the animal type of hemolytic streptococci. None were mucoid like *S. epidemicus*. The hemolytic zones were of the beta type measuring 2 to 4 mm. across. In tests for fibrinolysis all were negative for oxalated human blood. Eleven of the 22 hydrolysed sodium hippurate, 13 fermented trehalose, 9 did not; only one fermented sorbitol, 21 were nega-

tive. Acid production was high, varying from pH 4.5 to 4.8. Five of 11 strains killed mice injected intraperitoneally in doses of 0.5 cc. of broth culture.

Conclusions. (1) Hemolytic streptococci are constantly present in the tonsils of apparently normal dogs (92.3%). They occur in predominant numbers in acute tonsillitis of dogs and are secondary invaders in the bronchopneumonia of distemper. (2) Experimentally acute tonsillitis is produced by swabbing freshly isolated canine strains on the surface of the tonsils of dogs. A human strain (*S. epidemicus*) failed to produce a reaction. (3) The streptococci from dogs conform to the animal type and differ from the human type.

8676 P

Combined Staphylococcal Endo- and Exo-Antigen.

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The pathogenic activity of microorganisms may be due to toxic substances of either cellular or extracellular nature or both. These substances are usually designated endotoxin and exotoxin, although it is likely that no sharp division line exists.

An ideal antigen for active immunization in the case of invasive microorganisms, which produce both endo- and exotoxin, should contain both endo- and exotoxic principles. Likewise, an ideal antibody-solution when administered therapeutically should be able to neutralize both the endo- and exotoxin of the homologous organism.

Staphylococcus pyogenes produces a soluble exotoxin¹ which is readily converted into formol-toxoid, and is used for active immunization of laboratory animals and human beings. The staphylococcus contains endotoxin,² and vaccines have been used for a long time in the prophylaxis and treatment of localized staphylococcal infections. Bacteriophage-solution is also used therapeutically, both on account of its lytic activity towards the organisms and be-

¹Burnet, F. M., *J. Path. and Bact.*, 1929, **32**, 714; 1931, **34**, 471; Leonard, G. F., and Holm, A., *J. Immunol.*, 1935, **29**, 209.

²von Lingelsheim, *Aetiologie u. Therapie d. Staphylokokken Infektion*, Berlin-Wien, 1900; Borrisow, P., *Beitr. z. path. Anat. u. z. Allgem. Path.*, 1894, **16**, 432.

³Larkum, N. W., *Am. J. Pub. Health*, 1933, **23**, 1155; *Proc. Soc. Exp. Biol. and Med.*, 1933, **30**, 1395.

cause bacteriophagic solution of the lysed staphylococcus is antigenic.⁸ The toxoid, the vaccine, and the bacteriophagic solution may be considered as representing different antigenic groups.

If an antigen embodied all these antigenic principles, the immunity would presumably be more effective than specific antitoxic immunity or a strict antibacterial immunity, and the administration of an antitoxin which in addition contained antibacterial antibodies would better match the toxin-producing and invading characteristics of *S. pyogenes*. Prompted by these considerations we prepared a multivalent staphylococcal toxoid which contains endotoxic as well as exotoxic principles of staphylococci.

Lysis by bacteriophage was preferred to other methods in order to avoid denaturation and because the antigenic activity of bacteriophage solution is well established.⁸

A detailed description of the method of preparation will be published later but the general outline is as follows: Inoculation of a Berkefelded staphylococcal toxin with pooled cultures of the different strains used in the preparation of the toxin; addition of an adapted bacteriophage; addition of formalin and merthiolate after clearing of the culture by the bacteriophage; incubation for conversion of the toxins into toxoid; Berkefeld-filtration and testing for innocuity and antigenicity in the usual manner.

In 3 experiments on 54 rabbits, we compared the antigenic potency of the staphylococcal bacteriophage-toxoid with a toxoid derived from the same staphylococcal toxin, and with a fresh bacteriophagic solution prepared in the same medium and with the same strains of staphylococci that had been used for the bacteriophage-toxin.

When the data for the 3 experiments were combined, it was found that the mean antitoxin-titers after inoculation with the 3 antigens were: Bacteriophage-toxoid, 7.3 units; staphylococcal toxoid, 4.3 units; and bacteriophage-solution, 0.56 units.

It has been claimed that resistance in bacteremias caused by the staphylococcus depends upon the titer of serum-antitoxin, but other factors may also be of importance as indicated by the results of the intravenous injections of 5 billion live virulent staphylococci into 2 groups of the immunized animals.

Of 15 rabbits which were injected with living organisms after immunization with staphylococcal toxoid, none survived more than 9 days, while 11 died within 48 hours. Of 14 rabbits immunized with bacteriophage-toxoid, 8, or 55%, survived the later injection of staphylococci. Finally, of 12 rabbits inoculated with

bacteriophage-solution, 3 or 25%, withstood the injection of live staphylococci. Horses have also been immunized with staphylococcal bacteriophage-toxoid for the production of a combined antiserum.

The procedure outlined may with minor changes be utilized with other microorganisms for the preparation of bacteriophage-toxoid, especially with those organisms that yield endo- as well as exotoxin and which may be lysed by bacteriophage. Thus it should be possible to obtain more effective antigens both for active immunization of human beings and for the production of more potent antibodies in the horse or in other animals.

8677 C

Determination of Ethyl Alcohol in Saliva.

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The concentration of alcohol in the blood and cerebrospinal fluid of man has for many years been accepted as an index of the state of his intoxication. There is often, however, difficulty in obtaining blood or cerebrospinal fluid from individuals under the influence of alcohol; and it is therefore more advisable to use, when possible, a more accessible and equally trustworthy index.

Friedmann and Brook¹ have recently shown that saliva also contains ethyl alcohol. We have performed a series of determinations on this fluid to see whether or not the concentration of EtOH found in it has some simple relationship to that found in a corresponding blood sample. The methods (macro and micro) used in this study have been described;² we should like to point out that in the following cases the results of the two methods agreed within $\pm 3\%$.

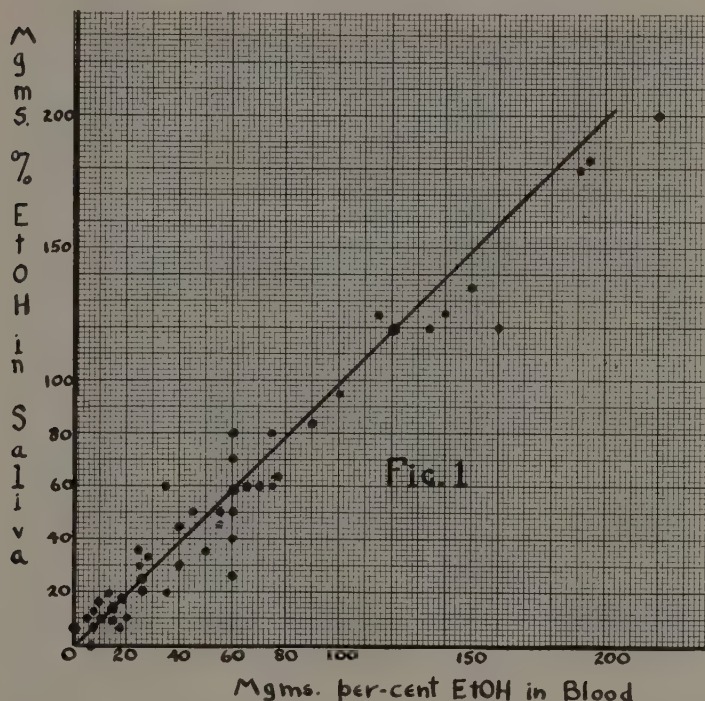
In 30 cases of normal individuals the saliva EtOH concentration was 0 mg. %, while the blood alcohol values of this group were from 0 to 7 mg. %.

Similar determinations in 50 cases of individuals in various states of intoxication showed satisfactory correlation of the EtOH concentrations in the saliva specimens with those in the corresponding

¹ Friedmann, T., and Brook, T., *Proc. Am. Soc. Biol. Chem.*, 1936, **30**, xxxvii; *J. Biol. Chem.*, 114.

² Abels, J. C., *Proc. Soc. Exp. Biol. and Med.*, 1936, **34**, 4.

bloods. The results of these experiments are perhaps best indicated in Fig. 1. There is an average deviation between the 2 of



± 10 mg. % EtOH; however, when an attempt is made to associate the blood and saliva alcohol concentrations with four arbitrary degrees of intoxication,² the following table can be set up:

TABLE I.

No. Cases	Symptom Group	— Blood Group —				— Saliva Group —			
		I	II	III	IV	I	II	III	IV
25	I	23	2	0	0	24	1	0	0
13	II	1	12	0	0	3	10	0	0
10	III	0	0	9	1	0	1	9	0
2	IV	0	0	0	2	0	0	1	1

Blood and saliva Group I correspond to 0-50 mg. % EtOH; Group II to 50-90 mg. %; Group III to 90-185 mg. %; and Group IV to more than 185 mg. %.

I am indebted to Dr. I. Greenwald for having suggested this problem and for his continued kind interest in the work. I also wish to express my gratitude to Dr. N. Jolliffe for his sincere co-operation.

Anesthetic Properties of Sodium-Ethyl-Pentyl, Malonyl-Thiourea.*

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It has been demonstrated that organic compounds which are related spatially as to the arrangement of the carbon atoms about a nonspecific central nucleus have similar pharmacological activities. Onium compounds formed about such atoms as nitrogen, sulphur, arsenic, phosphorus and antimony have properties best represented by the most active member of this group, acetyl choline (Dale and Burn,¹ Hunt²). Erlenmeyer and Berger³ demonstrated the immunological equivalence between =O, =NH, and =CH, in certain "determinant groups." There is also a probability that the amino group in the so-called sympathomimetic amines can be replaced by -Cl, (Mulinos and Osborne⁴).

The present study is concerned with a compound in which the =O group in the urea molecule of pentobarbital has been replaced by sulphur. The new compound possesses much of the action of the barbiturate. We report here a comparison between the 2 drugs, with the intention of emphasizing the increasing importance played by the spatial arrangement of atoms, as distinguished from the specific pharmacodynamic power of the individual elements constituting the compound.

Pentothal-sodium, Abbott No. 8060, is an amorphous, greenish white powder, having a strong sulphurous odor. It dissolves readily in water, to form a 10% solution, which has a distinctly green color, and a very disagreeable odor. The sulphurous odor diminished rapidly, to become almost imperceptible within a week, during which time the solution seemed to have lost but little of its potency. The 10% solution is strongly alkaline to litmus, it has a pH of 10.6, and forms precipitates with acids, and salts of silver.

Our experiments were performed upon rats and cats. Studies on respiratory exchange were performed on urethanized rabbits.

* Abbott No. 8060, obtained through the courtesy of Dr. T. D. Buchanan, of the New York Post-Graduate Medical School and Hospital.

¹ Dale and Burn, *J. Pharm. Exp. Therap.*, 1914, **6**, 147, 417.

² Hunt, *J. Pharm. Exp. Therap.*, 1925, **25**, 315.

³ Erlenmeyer and Berger, *Biochem. Z.*, 1932, **255**, 429.

⁴ Mulinos and Osborne, *Proc. Soc. Exp. Biol. and Med.*, 1935, **32**, 1344.

TABLE I.
Summary Comparison Between Pentobarbital and Pentothal. All Doses Administered Intraperitoneally.

Animal	Pentobarbital Sodium	Effect	Pentothal Sodium.
Rabbits	40-50 mg. x Kg.	Anesthesia.	60-70 mg. x Kg. 6-12 hrs.
Cats	40-45 " " "	" "	60-70 " " " 18-36 "
Rats	40-60 " " "	" "	60-80 " " " 8-24 "
" "	120-150 " " "	" "	90-100 " " " 9 of 15 died.
" "	(0.64 mole x Kg.)	M.L.D. (Respiratory death).	(0.43 mole x Kg.) (See also Tatum ⁹).
Rabbits	0.36 in moles x Kg.	M.E.D./M.L.D.	0.70 in moles x Kg.
" "	Depressed, with marked potentiation with morphine and heroine.	Respiration.	Depressed with marked potentiation with morphine and heroine.
Rabbits	None. (See Lieb and Mulinos ⁵).	Vagus paralysis.	None.
Cats	Sneezing and coughing marked during induction.	Respiratory tract.	Slight sneezing and coughing during induction.
Rabbits	None.	Irritation.	None.
Cats	102 Mg. %, av. of 97 cats. (See Mulinos ⁶).	Blood sugar.	94 mg. %, av. of 11 cats.
Rats	Depressed. (See also Gruber ⁷).	Isolated uterus and intestine.	Depressed.
Rabbits	Definite in over one-half of animals. (See Barris and Magoun ⁸ and Mulinos ⁶).	Reducing subst. in urine.	Traces in about one-third of animals.
Guinea Pigs	Slit pupil. (See Mulinos ⁶).	Pupiloconstriction.	Slit pupil.
Cats	Marked	Relaxation of the nictitating membrane.	Marked.
" "		Analgesia.	Poor.
Rats	Poor.		
Rabbits			
Cats			

⁵ Lieb and Mulinos, *Proc. Soc. Exp. Biol. and Med.*, 1929, **26**, 709.

⁶ Mulinos, *Arch. Internat. Pharm. et Therapie*, 1934, **47**, 111.

⁷ Gruber, *J. Pharm. Exp. Therap.*, 1936, **56**, 341.

⁸ Barris and Magoun, *Proc. Soc. Exp. Biol. and Med.*, 1932, **29**, 684.

⁹ Tatum, *Am. J. Surgery*, 1936, **31**, 464.

A Thyrotropic Field Surrounding the Immature Pituitary of the Tadpole.*

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It was previously reported that pituitary primordia when grafted are capable of developing thyrotropic activity in the tadpole, though such activity is generally delayed in appearance and much less than that of the normal gland.^{1, 2} In the course of a series of investigations on the development of function in such grafts, it was found that multiple grafts placed under the adhesive discs and into the eye cup of hypophysectomized tailbud embryos of *Rana sylvatica* induced precocious metamorphosis in the host. Since single grafts similarly placed did not induce precocity, it was considered that the excess pituitary tissue was responsible for the precocity.³ No precocity was observed by Blount in *Amblystoma* with excess pituitary grafts.⁵

In attempting to confirm these observations in *R. pipiens*, a series of normal animals with extra pituitary primordia inserted into the eye, near the otocyst and under the adhesive discs was prepared. Only those animals which had extra pituitaries placed under the adhesive discs showed precocity. In another experiment one animal which had its own pituitary primordium placed under the adhesive discs, displayed unmistakable metamorphic precocity.

It was thought that the explanation of these results might lie in some effect of the proximity between the pituitary and thyroid, for when grafts are placed under the adhesive discs, they develop in the neighborhood of the thyroid gland. The high proportion of precocity observed in tadpoles with several grafts in this region as compared to those with one, may be accounted for by the greater depth to which the grafts must be pushed when several are inserted, for embryological considerations indicated that the more deeply placed the grafts are, the closer they approach to the place of origin of the thyroid. The following experiment was conducted to test this hypothesis.

* This research has been supported in part by the Josiah Macy Foundation.

¹ Etkin, William, *Anat. Rec.*, 1934, **60**, Suppl. 1; 1935, **64**, Suppl. 1.

² Atwell, Wayne J., *Proc. Soc. Exp. Biol. and Med.*, 1935, **33**, 224.

³ Etkin, William, *Proc. Soc. Exp. Biol. and Med.*, 1935, **32**, 1653.

⁵ Blount, Raymond F., *Proc. Nat. Acad. Sci.*, 1930, **16**, 218.

Experiment 1936—3. *Rana pipiens*—All animals from one batch of eggs. *Operations*:—Type A—175 tailbud embryos were hypophysectomized and their own pituitary primordia inserted under the adhesive discs and pushed forward to rest near the place where the thyroid is to form.

Type B—50 normal tailbud stages with thyroid primordia which had been dissected out of embryos 1-2 days older and inserted near the pituitary primordium. Such animals retained their own thyroids in the normal situation.

Type C—40 normal control animals.

All the animals were set out into fingerbowls, 4 animals to a dish with about 100 cc. of water, and fed spinach and oatmeal. They were kept on a water table surrounded by running water regulated to 26°-29°C. Of the Type A animals only those were set out which showed the darkening which is the effect of a successful take of the pars intermedia of the pituitary.

At 20 days after the operation most of the animals had reached a total length of 20-30 mm. A selection of these animals considered to be of interest were then fixed in Bouin's fluid and the appropriate regions sectioned serially, stained with Mallory's connective tissue stain and examined.

Results:—Controls—The data on the 40 control animals of this experiment were combined with 131 available in all other experiments with this species under the same conditions. The hind leg length was plotted as ordinates against the total length as abscissæ. This graph showed the range of variation in normal controls. In this way standards were established for the observation of early precocity in the hind leg growth and, (since hind leg growth depends upon the thyroid hormone) for estimating the degree of activity of the thyroids under the influence of the pituitary. Those animals in which the hind leg length was twice or greater the maximum size found in the 171 normals at a like total length were deemed definitely precocious, those showing an increase of 25% to 100% over the maximum in the controls were considered as probably precocious, and the rest regarded as not precocious (Fig. 1). Several animals of each operated type were allowed to continue their metamorphosis, which they did. These will not be considered in the present report.

Type A—Hypophysectomized animals with own pituitary placed near thyroid. Of 60 of these which were set out in the experiment, 14 were definitely precocious in hind leg growth, 6 were

FIG. 1



FIG. 2

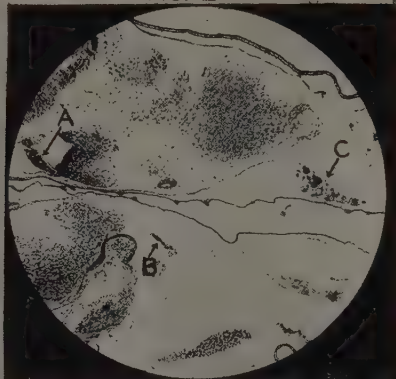


FIG. 3

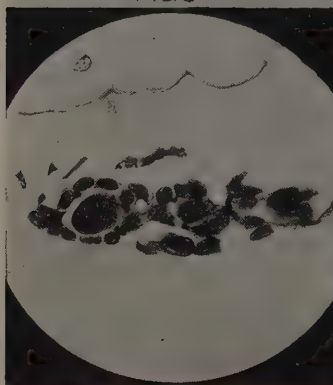


FIG. 4

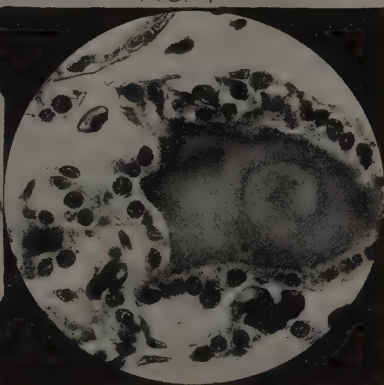


FIG. 1.

Above—Normal control—Total length, 26 mm. Hind leg length, 0.2 mm. Arrow points to limb bud showing normal size, appears as white dot.

Center—Hypophysectomized with own pituitary implanted near thyroid. T. L., 25; H. L. L., 2.0.

Lower—Normal animal with extra thyroid primordium implanted near hypophysis. T. L., 26; H. L. L., 1.5.

FIG. 2.

Sagittal section of head of Type B showing normal hypophysis at "A," normal-site thyroid at "B" and thyroid graft at "C." Section selected to show the maximal cross-section size of normal thyroid. T. L., 27; H. L. L., 0.6. Mag. about 29 $\times$. Note that the mouth cavity separates "A" from "B".

FIG. 3.

Largest section of normal thyroid of Type B animal. T. L., 27; H. L. L., 1.4, Mag. about 420 $\times$

FIG. 4.

Section through grafted thyroid of same animals as Fig. 3. Entire section not shown. Mag. about 420 $\times$.

probably so, and in the rest the hind leg length lay within the normal range of variation.

Type B—One extra thyroid placed near the pituitary. Of 49 of these set out, 8 were definitely precocious, 5 were probably so, and the rest were normal.

Histological Findings:—The development of the thyroid in this and related species was studied previously. It was shown that before metamorphosis, the gland is extremely small, the follicles being but a few times larger than the diameter of a nucleus, and with very flat epithelium, poor in cytoplasm (Fig. 3). Such glands were found in 3 normal controls and, most significantly, in all the normal-site glands in the Type B animals. In the Type A animals, 7, classified as definitely precocious, showed thyroids greatly enlarged in total size, most follicles much larger than normal. In some cases very small colloid vacuoles were shown. Often one follicle presented more area than the largest x-section of an entire normal gland. The epithelium was decidedly higher than in controls, varying from low cuboidal through columnar. The gland was characteristically highly vascularized. The picture resembled that of mid-prometamorphosis in normal development, but the gland did not attain the extreme activity shown by normal thyroids at the climax of metamorphosis. Figure 4 shows the type of epithelium which characterizes such glands. These will be designated as activated glands. In 2 classified as probably precocious, one activated gland, and one about normal were found. Of 5 classified as not precocious, 2 activated, one intermediate and 2 small normal glands were found.

In all cases the graft hypophysis was found in the neighborhood of the thyroid, sometimes in contact with it. It is not apparent that the cases with non-activated thyroid showed any greater separation of the glands than did the others, though this factor might contribute. In several cases where the thyroid was diffuse in structure, it appeared that the part of the gland closest to the graft seemed to have the most highly activated epithelium, though quantitative distinctions cannot be relied upon to any great extent.

In the Type B animals sections are available of 4 specimens in each classification, precocious, probably precocious, and not precocious. With the exception of one animal in the last group all these showed that the graft thyroids placed near the hypophysis were decidedly enlarged as compared to the glands in the normal position (Figs. 2, 3, 4). The epithelium in all except 3 cases in the

latter 2 classifications was clearly activated (Figs. 3, 4). In one non-precocious animal no graft thyroid was found. Thus the normal thyroid glands of these animals showed the inactive condition characteristic of the gland in the normal tadpoles of this age whereas the grafted thyroid is clearly increased in size and activity. That the mere grafting of thyroids does not result in activation has already been shown by Allen⁴ and others.

The graft thyroids were rather widely distributed, several lying in contact with the anterior part of the forebrain, some in the basicranial cartilage, but in all cases touching or lying in a space that borders the meninges lining the cranial cavity (Fig. 2).

These results are interpreted as indicating that approximation of these glands brought about by the transplantation of either one induces a precocious activation of the thyroid. This activation was morphologically apparent in nearly all cases and physiologically demonstrated by precocious hind leg growth in most cases. The absence of metamorphic activity in some instances where the thyroid appeared activated morphologically may be attributed to the early stage at which the animals were sacrificed.

It is thought that this experiment demonstrates that the pituitary of an immature animal produces and releases into its neighborhood effective concentrations of thyrotropic hormone. Such secretions are somehow rendered ineffective for the normal thyroid whether by reason of dilution in the blood stream or otherwise.

It would appear from this experiment that the precocity observed with multiple pituitaries as previously reported³ was due to the position of the grafts and not to the excess tissue *per se*.

8680 C

Effect of Various Amino Acids on Motility of Excised Segments of the Small Intestine.

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In the course of some unpublished work by Weinstein and Rettger on the effects of alkaline bacterial cultures it was deemed ad-

⁴ Allen, B. M., *Anat. Rec.*, 1929, **44**, 207; *Contributions to Marine Biology*, Stanford U. Press, 1930.

visible to determine whether or not a depressant action on intestinal motility which had been noted was caused by the presence of amino acids in the cultures. Tests were therefore made using pure amino acids to determine whether an effect similar to that of the bacterial cultures could be elicited.

Hirschfelder and Cantwell¹ reported an investigation on the effect of various amino acids on the movements of excised segments of intestine of cats and rabbits. Using aspartic acid, glutamic acid, glycoll, tyrosine, histidine hydrochloride and cystine in M/100 concentration, they were able to demonstrate a depressant effect. This inhibition, however, was correlated with a lowering of the pH of the medium in which the intestine was allowed to contract. Asparagin was found by these investigators to exert a slightly stimulating action.

In the present study cats were used as the experimental animals. They were killed by stunning and pieces of the duodenum, jejunum and ileum immediately removed and immersed in Ringer-Locke solution. The segments were kept in the ice box until used, although no piece of intestine was preserved longer than 48 hours. In carrying out each experiment the intestinal segments were immersed in a bath of oxygenated Ringer-Locke solution. The purest amino acids obtainable were used and were added to the suspending medium in such amounts as to give known concentrations. Determinations were made of the pH of the Ringer-Locke solution before and after adding the amino acids. All of the experiments were repeated on intestinal segments from at least four cats.

Aspartic and glutamic acids in a dilution of 1:2,000 were found to depress the tone and movement of segments of the duodenum, jejunum and ileum; the molar concentrations of these acids were M/266 and M/294 respectively. Alanine and phenylalanine in dilutions of 1:1,000 (M/89) and 1:2,000 (M/330) respectively were found to exert only a slight temporary decrease in tone and rhythm of the intestine. This inhibition was quickly followed by a return to the normal type of contraction. Tyrosine in 1:2,000 (M/362) was without any effect. Leucine in a dilution of 1:5,000 (M/655) and tryptophane (1:2,000 or M/408) caused a slight increase in tone and rate of contraction of the excised segments of duodenum, jejunum and ileum.

Summary and Conclusions. The results with aspartic and glutamic acids are the same as those reported by Hirschfelder and

¹ Hirschfelder, A. D., and Cantwell, W., *J. Pharm. and Exp. Therap.*, 1918, **11**, 178.

Cantwell even though much weaker solutions were used in our experiments. Their explanation, that the depressant action is probably due to a decrease in pH was checked and found to hold also for the present work. Alanine and phenylalanine exerted only a slight temporary depressing action despite the fact that the molar concentration of the alanine was much higher than that of either aspartic or glutamic acid.

Since the bacterial cultures used in the previous work by Weinstein and Rettger were alkaline in reaction and the amino acids, when neutralized, had no effect on intestinal motility, as judged by experiments of the type here described, it must be concluded that the depressant action of the bacterial cultures was not due to their content of amino acids.

8681 P

Effect of Tannic Acid on Intranasal Infection with Pneumococci.

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It has been shown^{1, 2} that certain viruses are infective for mice when instilled into the nares and that this capacity to invade the body of the mouse through the mucosa of the nose can be greatly diminished by preliminary intranasal treatments with tannic acid or alum-solutions. It has also been shown³ that certain strains of pneumococci are infective for mice by the nasal route and that, under the conditions of the experiment, invasion takes place both through the nasal mucosa and through the pulmonary alveolar walls.⁴

The present experiments were planned to test whether this nasal infectivity of pneumococcus strains is modified by preliminary tannic-acid treatments. For this purpose, 5 experiments were undertaken using groups of young (3 to 6 weeks old) Swiss mice. In each experiment 15 mice were treated with weak solutions of tannic acid for 3 or 4 days before the infecting inoculation was

¹ Olitsky, P. K., and Cox, H. R., *Science*, 1934, **80**, 566.

² Armstrong, C., *Pub. Health Rep.*, 1935, **50**, 43.

³ Webster, L. T., and Clow, A. D., *J. Exp. Med.*, 1935, **58**, 465.

⁴ Rake, G., *J. Exp. Med.*, 1936, **63**, 191.

made. In 2 cases, 0.7 or 1.0% solution of tannic acid in normal saline was used and in the other 3, 0.8% solutions of tannic acid in 1.0% glycerol. In the first test, 7 preliminary treatments, each of 0.03 cc. of the tannic acid, were given intranasally; in the second, 9, in the third, 6, and in the last 2, 8 similar treatments. Fifteen mice remained untreated as controls in each test.

Cultures of strains of pneumococci of both Type III and Type XIX, known to be intranasally virulent, were used. In the past the use of intranasally virulent strains of pneumococci has been attended with the difficulty that this virulence or invasiveness, which is independent of the intraabdominal virulence is apt to be lost suddenly and without apparent reason during subculture or on animal passage³ and cannot be regained by any of the means so far adopted. It has now been found, however, (Rake—unpublished) that this intranasal virulence can be maintained over long periods of time in cultures which have been rapidly frozen and dried *in vacuo*. Such cultures have been used in this investigation and in one case the culture was proved to be as virulent intranasally after being frozen and dried for 432 days as it had been prior to freezing and desiccation.

For the investigation 12-hour broth cultures of the heart's blood of a mouse dying from an infection with the strain required were used. Each mouse in both treated and untreated groups of the first 3 tests received 0.03 cc. of undiluted 12-hour culture in pneumococcal broth at pH 7.8. In the other tests the culture was centrifuged rapidly to throw out most of the organisms which were then resuspended in $\frac{1}{3}$ the volume of broth. Only 0.01 cc. of this was given intranasally to each mouse to avoid, as far as possible, flow to and absorption through the lungs. The mice were observed, the times of death noted, and the cause of death verified by autopsy and culture.

No significant difference could be shown between treated and untreated groups. Thus, in Experiment 1, using Type XIX, 10 of 15 treated mice and 9 of 16 controls died. In 2 other experiments with a Type III culture, 7 of 15 and 9 of 14 treated mice died, as compared with 8 of 15 and 10 of 15 controls. In the last 2 tests using smaller amounts of Type III culture, 9 of 30 treated mice died, as compared with 13 of 33 controls.

It was also possible to show by use of the technic already described⁴ that there was no demonstrable difference in the speed with which invasion of the blood-stream occurred in the treated and untreated groups of mice. In both groups invasion occurred within a very few minutes.

It seems, therefore, that the tannic-acid³ treatment, which is believed to act principally on the mucosa¹ of the nose, does not influence in any way the course of at least one intranasal bacterial infection. It should, however, be emphasized that pneumococci are believed to invade the body also through the pulmonary alveoli after intranasal instillation.⁴

8682 P

Concentration and Purification of the Gonadotropic Substance in Urine of Ovariectomized and Post-Menopausal Women.*

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While attempting to concentrate the gonadotropic activity of the urine of post-menopausal (or castrate) women, it has been found that the active material may be practically quantitatively concentrated into relatively non-toxic extracts by the use of tannic acid. The method developed for the precipitation and partial purification of the gonadotropic material is as follows.

The chloroform-preserved urine is chilled, siphoned from any sediment and acidified to pH 5 with acetic acid. For each liter of urine, 20 cc. of a freshly prepared aqueous 10% tannic acid solution are added. An immediate precipitate forms. After thorough mixing, the precipitate is allowed to settle in the cold room and is then collected at the centrifuge. The bulky precipitate, which contains considerable water, is extracted once with at least 5 parts of 95% ethyl alcohol followed by 3-4 extractions with 80% alcohol. Alcohol is removed by several washings with acetone and the residue is freed of acetone by reduced pressure. In addition to removing considerable pigment and other inert material, the alcohol-acetone treatment removes any estrogenic substances which may be present.

The resultant powder (100 to 200 mg. per liter of urine) con-

* A portion of this work was reported before the American Society of Biological Chemists at Detroit, April 13, 1935 (*J. Biol. Chem. (Proc.)*, 1935, **109**, liv.)

Aided by a grant, administered by Dr. P. E. Smith, from the Committee for Research in Sex, the National Research Council.

† Squibb Fellow, 1935-36.

tains 75-100% of the original gonadotropic activity of the urine† as indicated by parallel assays of the raw urine (or alcohol precipitates thereof). The activity of the dry tannate is quite stable, no loss being noted even after storage for more than a year at room temperature.

Assays are conducted by giving 20-22-day-old mice (8-12 gm. body weight) subcutaneous injections once daily for 3 days, the animals being sacrificed 72 hours after the first injection. Because with minimal doses ovarian weight alone is an unreliable criterion, the mouse unit has been tentatively defined as that amount which, when administered in the above manner, will cause vaginal canalization, at least a 200% increase in the weight of the uterus drained of fluid (controls 4-7 mg.), and a slight increase in ovarian weight as compared to uninjected controls. Such a unit is very sensitive and in the absence of estrogenic substances is quite accurate. We have never been able to demonstrate the presence of any estrogenic activity in the alcohol washed tannates.

Partial purification of the crude tannate is accomplished as follows. The dry tannate is thoroughly extracted 4-5 times with a barium hydroxide-barium acetate mixture at pH 9.0-10.0. Each extract is immediately adjusted to pH 7.0-8.0 with dilute H_2SO_4 and the combined solutions are freed of excess barium by addition of $(\text{NH}_4)_2\text{SO}_4$ solution, dilute ammonia being added, if necessary, to maintain the pH at 7.0-8.0. The barium sulphate is collected and leached several times with small volumes of dilute NH_4OH . The combined extract and washings are chilled, adjusted to pH 9.0-10.0 by cautious addition of ammonia water and immediately precipitated by the addition of 2-3 volumes of cold acetone. After chilling over night, the precipitate is collected at the centrifuge, washed with acetone and freed of acetone by reduced pressure. The product is an almost white powder which is completely soluble in water. Such powders retain 70 to 100% of the activity of the original tannate but only 15 to 25% of the original solids.

† Application of this precipitation to other types of urine (pregnancy, normal women, normal men) has yielded excellent results. Hellbaum *et al.*¹ have reported similar experience with pregnancy urine. Others^{2, 3, 4} have applied our method to normal urines with confirmatory results.

¹ Hellbaum, A. A., Fevold, H. L. and Hisaw, F. L., *Proc. Soc. Exp. Biol. and Med.*, 1935, **32**, 1566.

² Thomsen, O., and Pedersen-Bjergaard, K., *Compt. rend. Soc. d. Biol.*, 1935, **120**, 1143.

³ Pedersen-Bjergaard, K., *Zentralbl. f. Gynak.*, 1936, **60**, 372.

⁴ Friedman, M. H., and Weinstein, G., *Proc. Am. Physiol. Soc.*, 1936, p. 54.

More than half of the remaining inert material may be eliminated, with little or no loss of activity, by the following procedure. The powder is dissolved in 60% ethyl alcohol at pH 9.0-10.0 (ammonia water drop by drop). The somewhat turbid solution is then brought to pH 6.5 by the dropwise addition of glacial acetic acid. The precipitate which forms is immediately removed by centrifugation and is washed several times with small volumes of slightly acidified 60% alcohol. The clear brown alcoholic solution and washings are combined and an equal volume of acetone is added. The mixture is chilled over night, the precipitate collected, washed with acetone and freed of acetone by reduced pressure. The product is a white powder (10-15 mg. from each liter of pooled urine), readily and completely soluble in water and active in mice in total doses of 0.10-0.15 mg. Solutions in which the original activity of the urine is concentrated as much as 10-20 thousand times are easily prepared.

This material produces no toxic effects even when administered in very large doses. Hypophysectomized immature rats, which are extremely sensitive to toxins, have shown no ill effects when injected with as much as 1,000 mouse units (equivalent to 5-10 liters pooled menopause urine) over a period of five days.

These preparations contain no tannic acid but are completely precipitated from acidified solution by the addition of tannic acid. However, no precipitation is obtained with the other alkaloidal reagents except phosphotungstic and phosphomolybdic acids. This may explain why tannic acid is much more efficient than are the other protein precipitants in the initial precipitation of the activity from the urine.

8683 P

Inhibitory Effects by the Hypophysis of Rats Following Injections of Pregnant Mare Serum Hormone.*

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Regarding the reciprocal pituitary-gonad relationship, most investigators agree that prolonged treatment with estrogenic substances inhibits the secretion of gonadotropic hormones from the hypophysis. Møller-Christensen¹ and du Shane *et al.*,² working with rat parabiosis, have found that the hypophysis exerts an inhibitory effect on overstimulated ovaries. The experiments here reported are harmonious with these findings in parabiotic rats.

1. *Controls.* Both normal and spayed (2-17 weeks) adult female rats were bled and the serum separated by coagulation. The gonadotropic action of the serum was tested by injecting 1 cc. of rat serum, in 5 divided doses, into immature female mice. The ovaries of the mice were weighed 96 hours after the first injection. As may be seen from the table, the normal rat sera (29 rats in 31 mice) contained no detectable gonad-stimulating hormones and the castrate rat sera (29 rats in 27 mice) but small amounts.

2. *Treated rats.* Similar rats, normal and spayed (2-17 weeks), were then injected with 50 mouse units daily of a gonadotropic hormone from pregnant mares' serum ("Antex" Leo‡). Following 9 to 86 days' treatment, the rats were sacrificed 24 hours after the last injection and the serum tested on mice. The treated normal rat sera (50 rats, 49 mice) gave slightly positive responses whereas the treated spayed rat sera (32 rats, 33 mice) contained about 5 times as much of the gonad-stimulating factor (Table). There was no apparent relationship between the length of treatment of

* Aided in part by a grant from the P. Carl Petersen Fund, Copenhagen, and by a grant, administered by P. E. Smith, from the Committee for Research in Problems of Sex, The National Research Council.

† Rockefeller Foundation Fellow.

¹ Møller-Christensen, E., *Acta Path. et micr. Scand.*, 1935, Suppl., 22.

² du Shane, G. P., *et al.*, *Proc. Soc. Exp. Biol. and Med.*, 1935, **33**, 339.

‡ "Antex" (manufactured by the "Løvens kemiske Fabrik," Copenhagen) is a purified gonadotropic hormone preparation from the serum of pregnant mares. One mouse unit is the minimum amount of hormone which will double the weight of the ovaries in 3-weeks-old mice.

TABLE I.
Gonadotropic Effect in Infantile Female Mice of 1 cc. of Serum from Donor Rats After Different Treatment.

Type of rat	Hormone treatment*	No. of rats	Aver. B.W. gm. at autopsy	No. of mice injected	Wt. of 1 mouse ovary in mg.		
					Aver.	Min.	Max.
Normal	None	29	146	31	1.1	—	—
"	Antex	50	146	49	1.8	1.1	2.7
Spayed	None	29	140	27	1.5	1.2	1.8
"	Antex	32	152	33	5.8	3.7	6.9
"	Antex + estrin	10	159	10	2.8	1.9	3.9
Hypsect.	Antex	9	127	10	5.4	2.7	8.3
" + spayed	"	12	127	14	6.5	4.4	9.6
" " " + estrin	"	9	123	9	5.9	4.0	9.2

*Dosage described in text.

the donor rat (9 to 86 days) and the gonadotropic effect of its serum.

To eliminate the possibility that the gonadotropic factor already present in the spayed rats might augment the injected material, and thus account for the greater effects obtained in the injected spayed rats, equal amounts of "Antex" were mixed with serum from untreated normal and spayed rats and tested on mice (142 mice). Spayed rat serum with "Antex" gave about 50% larger mice ovaries than normal rat serum with "Antex." Similarly, simultaneous injections of untreated spayed rats' serum and serum of normal "Antex"-injected rats gave also about 50% larger mice ovaries than the serum of normal "Antex"-injected rats alone (21 mice). Both of these augmentation reactions were much less, however, than the responses obtained from the serum of spayed rats treated with mare serum hormone.

3. *Hypophysectomized-treated rats.* By the use of hypophysectomized rats§ it is thought possible to eliminate altogether the presence of any but injected gonad-stimulating substance. Nine non-spayed and 12 spayed, hypophysectomized animals were treated in the same way as the non-hypophysectomized had been. The gonadotropic hormone content of the sera of the spayed and non-spayed groups was nearly identical. (The ovaries of the treated hypophysectomized rats were different in no significant manner from those of the treated normals.)

The figure summarizes this material. All 4 types of rats are

§ In 8 of the 30 hypophysectomized rats the microscopical examination of the capsules revealed small fragments of pituitary tissue, but as the serum of these rats did not differ in any respect from that of the complete ones they are not excluded from the experiments.

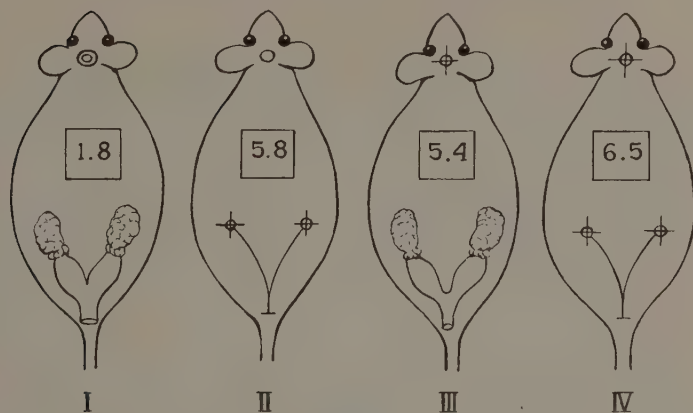


FIG. 1.

The gonadotropic potency of the serum of rats treated with gonadotropic hormone from pregnant mares.

- I. Normal.
- II. Spayed.
- III. Hypophysectomized.
- IV. " " -spayed.

"Antex"-treated. The amount of gonadotropic hormone in the serum is indicated by the number within the square (the *average* weight of *one* ovary from the test mice). The serum titre is high in the spayed rats, in the hypophysectomized and in the hypophysectomized-spayed rats, indicating that the difference between the normal injected and the spayed injected rats cannot be due to a hyperfunction of the castrate hypophysis or to a utilization of the injected hormones by the ovaries. Only in the presence of both organs (ovaries and hypophysis) is there a reduced amount of gonadotropic substance in the serum of rats treated with pregnant mare serum.

In normal, mare-serum treated rats the ovaries were markedly stimulated and the pituitary gland was greatly enlarged, as previously reported by Evans *et al.*³ On the other hand, there was no hypophysis weight increase in the spayed rats treated with "Antex." This suggested some action on the hypophysis by the stimulated ovaries and also suggested that such action and the resulting enlarged hypophysis might be correlated with the decreased serum titres, found only in the normal, treated animals.

4. *Estrin treated.* To test these suggestions, 10 spayed rats were treated with estrin ("Progynon B," 500 I.U. daily, 14 days) as well as with "Antex." Hypophysis enlargement was found, as

³ Evans, H. M. *et al.*, *Univ. Calif. Publications in Anat.*, 1934, **1**, 161.

in normal treated rats. Similarly, the serum of these animals also contained low titres of gonad-stimulating material, comparable to treated normal rats, with estrin producing ovaries. Nine hypophysectomized spayed animals, identically treated, gave high serum titres in spite of estrin injections (Table).

5. *Conclusion.* It appears that the hypophysis under the influence of estrin, whether supplied by injections or by overstimulated ovaries, actually inhibits possibly through the intermediary of some other endocrine gland the action of gonadotropic hormone.

8684 C

Transmission of Nutritional Muscular Dystrophy to Rabbits *in Utero.*

ALWIN M. PAPPENHEIMER AND MARIANNE GOETTSCH.

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When young rabbits are placed upon muscle-dystrophy producing Diet 11, the majority die within a period of several months.¹ Rarely the disease runs a protracted course; the animals may survive for a year, only to succumb eventually with characteristic degeneration of the skeletal muscles. One such adult dystrophic rabbit successfully completed gestation and gave birth to living young. This exceptional event, has permitted us to observe that nutritional muscular dystrophy may be passed on from mother to offspring.

Rabbit 241 was born in the laboratory on Jan. 15, 1935. On Feb. 19th, it was weaned and given Diet 11.* A biopsy from the thigh muscles was taken on Jan. 6th, 1936, when the animal had attained a weight of 2.4 kilos. A few necrotic fibers were found in each low power field. The muscle creatin was 540 mg.—within the normal range. Two subsequent biopsies on Nov. 19th and Dec. 11th showed little progression of the lesions.

¹ Goettsch, M., and Pappenheimer, A. M., *J. Exp. Med.*, 1931, **54**, 167.

* Diet 11. Rolled oats (Quaker).....355 parts
 Wheat bran (Pillsbury).....180 "
 Casein (Merek technical).....75 "
 Lard 80 "
 Cod liver oil (Mead-Johnson).....10 "
 NaCl 10 "
 CaCO₃ 15 "

On Jan. 7th, 1936, she was mated with a stock breeding male, which was known to have sired several normal litters, and which had been maintained on a stock diet of grains and alfalfa. On Feb. 8th, Rabbit 241 gave birth to a litter of 2. The young seemed somewhat scrawny, but they were not carefully examined for fear of disturbing the mother. The following morning, the newborn rabbits were found dead.

Their skeletal muscles at autopsy were strikingly pale. No other pathological change was recorded.

Histologically, sections taken from various muscles all showed extreme lesions (Fig. 1). Many of the fibers—in some field al-

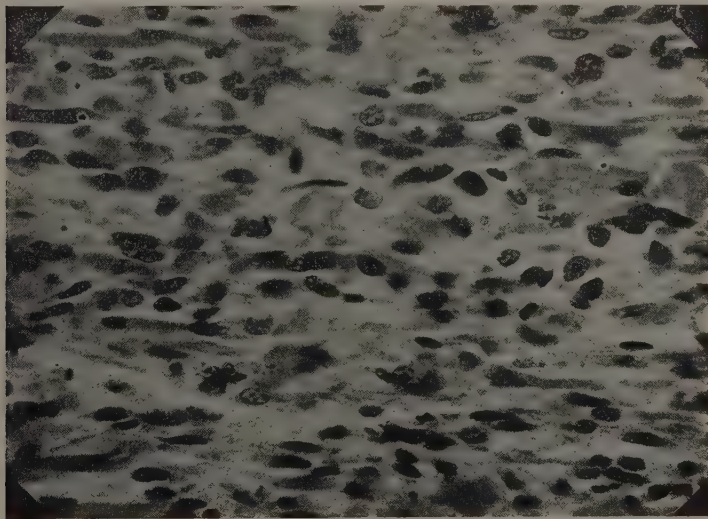


FIG. 1.

Rabbit 241 A. Section of thigh muscle showing necrotic fibers, myocytes and histiocytes. H-E. $\times 460$.

most 50%—were undergoing hyaline necrosis, with segmentation and rupture. They were widely separated by an oedematous stroma in which were great numbers of elongate fusiform cells with vesicular nuclei and basophilic cytoplasm. These were identified as myocytes. There were also many rounded histiocytes, and a few mature lymphocytes. The lesions were thus identical with those found in certain stages of adult nutritional muscular dystrophy.¹ As in the post-natal disease, the cardiac muscle was not affected.

Since these young rabbits lived but a day, these extreme muscular

lesions must have developed *in utero*. The further history of the mother is interesting. Following the birth of the young, she lost weight rapidly—about one kilo in 17 days. Towards the end, she ate but little, and there was great prostration with rapid wasting of the muscles. She was killed on Feb. 26th. At autopsy, the subcutaneous and intraabdominal fat was still preserved; the muscles were pale, streaky and devoid of contractility. The uterus was involuted. There were no incidental lesions of significance.

Microscopically, blocks taken from numerous muscles showed an advanced stage of dystrophy, with many recently necrotic fibers, as well as much interstitial fibrosis and fat replacement (Fig. 2).

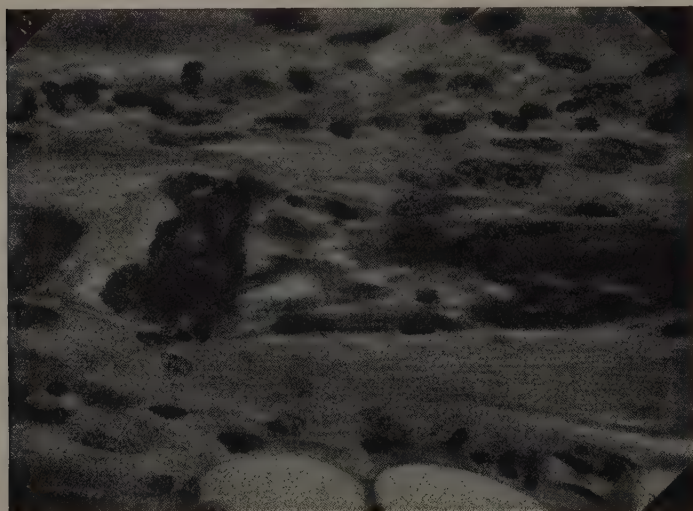


FIG. 2.

Rabbit 241. Mother. Section of adductor muscle, showing necrosis of fibers, multiplication of myocytes and histiocytes. H-E. $\times 460$.

The precise factors concerned in the production of nutritional muscular dystrophy of herbivora have not yet been clearly defined. It is therefore not justifiable at the present time to speak of this condition as a deficiency disease. But evidence is accumulating that the addition of vegetable oils (soybean oil,² cotton seed oil³) to the experimental diet exercises a protective effect. In our lab-

² Goettsch, M., and Pappenheimer, A. M., *Proc. Meetings Am. Soc. Exp. Biol.*, Washington, 1936, March 28 (In press).

³ Madsen, L., *J. Nutrition*. Personal communication.

oratory we have succeeded in raising animals to maturity and in keeping their offspring in apparent health by replacing the lard in Diet 11 by soybean oil. The protective factor must therefore pass to the fetus, because in its absence, the offspring are born with advanced muscular lesions.

8685 C

Effects of Synthetic Androsterone on Accessory Reproductive Organs of the Male Ground Squirrel.*

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From the Department of Anatomy, University of Missouri.

Accessory organs of immature, non-rutting adult and castrated male ground squirrels (*Citellus tridecemlineatus*) have been shown to respond greatly to injections of male hormone prepared from bull testes and human male urine.¹ Synthetic androsterone,‡ reported to stimulate male accessories in castrated birds and mammals,²⁻⁶ was administered to annual-breeding male ground squirrels in order to determine its effects on accessory reproductive organs during the season when testis hormone is released in minimal quantities, if at all, by gonads in normal males of this species.

The low state of reproductive organs in 25 normal males was evaluated by the following methods without the actual removal of tissues, in order to evade possible compensatory hypertrophy: (1) inspection of scrotal skin for pigmentation, (2) manual palpation of bulbar gland, (3) examination of epididymis and ductus deferens at exploratory laparotomy, and (4) measurement of the testis

* Aided by a grant from the National Research Council, Committee for Research in Problems of Sex.

† Much of the investigation reported in this paper was done in the Department of Zoology of the University of Chicago. The writer wishes to thank Dr. Carl R. Moore, who provided research facilities in his laboratory during the summer of 1935.

¹ Wells, L. J., *Anat. Rec.*, 1934, **60** (Suppl.), 53; 1935, **62**, 409.

‡ Prepared from cholesterol. Kindly furnished by Drs. Ruzicka and C. C. Haskell and the CIBA Company.

² Ruzicka, L., and Tschopp, E., *Schweiz. Med. Woch. Schr.*, 1934, **64**, 1118.

³ Callow, R. K., and Parkes, A. S., *Bioch. J.*, 1935, **29**, 1414.

⁴ Callow, R. K., and Deansely, R., *Bioch. J.*, 1935, **29**, 1424.

⁵ Korenchevsky, V., and Dennison, M., *Bioch. J.*, 1935, **29**, 1720.

⁶ Tschopp, E., *Klin. Woch.*, 1935, **14**, 1064.

through a peritoneal incision. Testis size, as an index of the hormonal condition of accessories, owes its reliability to the fact that the writer has considered gross and histological data (concerning testis and 6 accessory reproductive organs) for more than 250 normal males of this species killed at various stages of the sexual cycle since 1931.

Sixteen of these 25 animals were treated with androsterone, (dissolved in olive oil) while 9 were sacrificed as operated uninjected controls. Thirteen immature and 3 adult males were given from 0.5 mg. to 1.5 mg. of androsterone daily for periods ranging from 20 to 31 days, and were autopsied one day after the last injection. Sacrifice of experimental and control animals occurred during the interval from August 18th to November 6th; 84 other normal males killed between these dates since 1931 served as additional controls. Seminal vesicles, prostate gland, Cowper's glands and

TABLE I.
Gross Fresh-Weight of Accessory Organs.

		Treatment		Fresh weight in mg.					
Age	No.	Mg. daily	days	Date killed	Body wt. in gm.	Seminal vesicles	Prostate gland	Cowper's glands	Bulbar gland
Young									
	1040	0.5	20	8-18	110	6.8	3.8	6.2	30.0
	1041	0.5	20	8-18	97	11.8	4.0	8.4	32.2
	1042	1.0	20	8-18	120	8.6	3.2	8.2	41.4
	1043	1.0	20	8-18	100	13.6	4.0	8.8	23.4
	1044	1.5	20	8-18	138	11.8	4.0	16.2	35.2
	1045	1.5	20	8-18	90	16.4	6.2	20.2	44.0
	1046	Control		8-18	135	7.4	3.4	6.0	25.2
	1047	"		8-18	124	8.2	3.9	8.4	25.6
	1061	0.5	29	8-27	117	8.0	4.0	6.8	27.8
	1062	0.5	29	8-27	110	6.6	4.0	7.2	25.4
	1063	1.0	29	8-27	118	18.2	6.2	17.6	58.4
	1064	1.0	29	8-27	146	11.4	4.8	17.0	40.6
	1065	1.5	29	8-27	134	6.0	4.2	9.2	25.8
	1066	1.5	29	8-27	156	14.0	6.6	17.0	53.8
	1067	Control		8-27	100	7.0	4.0	8.2	31.8
	1068	"		8-27	157	9.0	4.2	6.0	25.8
	1082	1.5	31	11-6	190	10.0	4.6	19.1	37.0
	1081	Control		11-6	198	6.2	3.6	4.6	30.0
Adult									
	1022	0.5	29	8-27	164	35.0	22.2	35.8	177.4
	1023	1.5	29	8-27	222	52.8	30.0	69.6	194.2
	1020	Control		8-27	212	36.0	20.2	46.0	220.2
	1021	"		8-27	170	38.4	14.8	33.4	159.0
	1060	"		8-26	168	34.0	18.8	29.2	133.0
	1077	1.5	31	11-6	208	31.5	19.5	40.5	160.0
	1083	Control		11-6	250	21.0	12.6	25.6	172.8

bulbar gland of each animal were weighed while fresh; all of the above organs, together with epididymis and ductus deferens, were then fixed in Bouin's solution, sectioned at 6 micra and stained with Ehrlich's hematoxylin and eosin.

Gross weights of treated accessory organs ranged from values approximately equaling those for untreated glands to values twice as great as those for control organs (see table). In general, gross weights were largest following the higher doses and smallest after the lower doses. Stimulation was more noticeable in young males (small body-weight) than in adults.

Histological studies revealed unmistakable signs of stimulation of the epithelium of each accessory organ of treated males; however, the degree of stimulation was not great in any case. The epithelia of seminal vesicle, prostate gland, Cowper's gland, bulbar gland, epididymis and ductus deferens were measured with an ocular micrometer, and the average of 5 measurements for each organ from each of the 25 animals was recorded. Average measurements of epithelial height indicated that the magnitude of histological stimulation, in treated males, was approximately parallel to that of gross stimulation shown in the table. Other histological criteria, such as increased organization of the epithelium and increased granulation of epithelial cytoplasm, offered convincing evidence that androsterone treatments stimulated all accessory organs in each case (even though the stimulation was relatively slight).

Effects of synthetic androsterone on accessory organs in this species may be compared with effects of male hormone prepared from bull testes and male urine.¹ Quantitative comparisons can have no more than suggestive merit, since injected doses of these different substances were not identical in terms of capon units. However, the two following comparisons may be noted: (1) weight of accessory organs in males receiving male urine extract, in daily doses of 6 capon units for 30 days, was approximately the same as that in animals treated daily with 1.0 mg. to 1.5 mg. synthetic androsterone for a similar period; (2) daily injection of 6 capon units of bull testis extract, for a 29-day period, stimulated accessory organs much more than treatments with either male urine extract or synthetic androsterone for a similar period.

Lack of marked response of accessories in the ground squirrel to synthetic androsterone may be due possibly to the fact that the hormone was given in insufficient amounts; nevertheless, 1.5 mg. synthetic androsterone daily for 29 to 31 days proved effective in

stimulating the precocious formation of spermatozoa in both immature and non-rutting adult ground squirrels (4 cases).⁷

The value of the present study lies principally in the fact that synthetic androsterone proved capable of stimulating seminal vesicles, prostate gland, Cowper's glands, bulbar gland, epididymis and ductus deferens in a mammal other than those previously studied.

8686 P

An Experimental Study of Cardiopericardial Adhesions.*

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From the Laboratory of Surgical Research, Western Reserve University School of Medicine.

The generally accepted conclusion that pericardial adhesions play a fundamental rôle in the production of cardiac hypertrophy has been widely taught and approved.¹ Doubt was cast upon this established point of view by the many experimental operations on the heart and pericardium carried out during the past 12 years by Beck² and his associates. An additional reason for the study of this subject lies in the fact that the myocardium can be given a new blood supply through tissues grafted upon the heart. The Beck operation³ involves myocardial adhesions and the rôle of adhesions needs critical study if coronary sclerosis is to be treated by this new surgical principle.

Twenty-six dogs were divided into 2 groups. In one group the pericardium alone was attached to the dome of either diaphragm.

⁷ Wells, L. J., and Moore, C. R., *Anat. Rec.*, 1936 (in press).

* Read at the Joint Meeting of the Experimental Medicine Section of the Cleveland Academy of Medicine and the Cleveland Section of the Society for Experimental Biology and Medicine at the Institute of Pathology, March 13, 1936.

¹ Karsner, Howard T., *Human Pathology*, 4th ed., J. B. Lippincott Co.; Krumbhaar, E. B., Pericardial disease, in *Nelson Loose-Leaf Living Medicine*, vol. IV, p. 525; White, Paul Dudley, *Heart Disease*, Macmillan Co., 1931, p. 516.

² Beck, C. S., *Surg. Gynec. and Obst.*, 1935, **61**, 407; *Arch. Surg.*, 1929, **18**, 1659; *J. A. M. A.*, 1935, **104**, 714; Beck, C. S., and Griswold, R. A., *Arch. Surg.*, 1930, **21**, 1064.

³ Beck, C. S., *Ann. Surg.*, 1935, **102**, 801; Beck, C. S., and Tichy, V. L., *Am. Heart J.*, 1935, **10**, 849; Moritz, A. R., and Beck, C. S., *Am. Heart J.*, 1935, **10**, 874.

In the other group the myocardium and pericardium were attached to the diaphragm. The attachment was made well lateral to the central tendon of the diaphragm. This displaced the heart to either side. Attachment was always made so as to produce marked tension between diaphragm and pericardium. Various observations and studies were carried out on these animals over a period of $2\frac{1}{2}$ years.

Signs of circulatory embarrassment were not encountered in a single instance. The response of the animals to exercise tolerance tests was normal. The observation of greatest importance was that cardiac hypertrophy was not found either grossly or microscopically.†

This is borne out by the fact that the mean heart weight to body weight ratio in our series of dogs in which the heart was displaced to the left by the attachment of the pericardium alone was 0.00713; while the mean ratio for the group in which the displacement was to the right was 0.00696. With combined adhesions and displacement to the left the mean ratio was 0.00704, and was 0.00724 with combined adhesions and displacement to the right. These are within the limits set up for heart weight to body weight ratios by Herrmann.⁴ Microscopic sections from control animals and from animals in this series of experiments were subjected to objective observation and no significant alterations in transverse diameter were observed.

We also carried out a study of the autopsy records of the Lakeside Hospital from 1906 to 1929. In 2,600 autopsies there were 47 cases in which extensive pericardial adhesions were found. In 32 cases in which the hearts had undergone hypertrophy there was concomitant heart disease or vascular disease which in itself could account for the hypertrophy (agreeing with Chevers's⁵ unaccepted observations in 1842). In 15 instances extensive pericardial adhesions were found associated with hearts that were either normal or even smaller than normal in size. In none of these cases in which the heart did not show hypertrophy was there vascular, myocardial or valvular disease present. Almost without exception the largest hearts were the seat of rheumatic pancarditis. (In the earlier records the terminology used for the diagnoses is at times not so clear cut as our present-day terminology.)

† Dr. M. A. Simon of the Institute of Pathology carried out the microscopic study.

⁴ Herrmann, G. R., *Am. Heart J.*, 1925, **1**, 213.

⁵ Chevers, Norman, *Guy's Hospital Reports*, 15, 387; Oct., 1842.

In view of our experiments and the analysis of autopsy material, we are led to believe that pericardial adhesions *per se*—especially adhesions to the diaphragm—do not play a rôle in the production of cardiac hypertrophy.

8687 C

Further Studies on the Thermostable Antigen in Beef Cells.

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A thermostable alcohol-insoluble (B.T.) antigen has been demonstrated in beef- and sheep-erythrocytes.^{1, 2} In order to study further the nature of this antigen the production of specific antibodies in some experimental animal was desirable. Weil³ demonstrated the formation of beef- and sheep-lysins in guinea pigs immunized with boiled sheep-erythrocytes. Although Weil's work has been criticized on the ground that the thermolabile sheep-beef antigen was incompletely destroyed it now seems probable that the beef-lysins were engendered by the B.T. antigen in the boiled sheep-cells. In view of Weil's experiment, and since no B.T. antigen has been demonstrated in the guinea pig this species was selected for further study of the thermostable beef-antigen.

Guinea pigs were immunized by intraabdominal injections of 1.5 to 2.5 ml. of washed raw, boiled, or autoclaved beef-erythrocytes at 5-day intervals. Boiled cells were prepared by boiling a 25% suspension for 20 minutes. Autoclaved cells consisted of boiled cells further treated by autoclaving at 125°C. for 15 minutes. The pigs were bled from the heart and serum tested for lysins and agglutinins for beef-, sheep- and horse-erythrocytes. In the lytic tests .25 ml. of 1:20 guinea pig's complement and .25 ml. of one percent cell-suspension were added to tubes containing .5 ml. of serial dilutions of the immune serum. The degree of hemolysis was recorded after 30 minutes at 37°C. Agglutinative titers were determined by adding .5 ml. of .5% cell-suspension to .5 ml. of the serum-dilution and reading after 2 hours at 37°C.

¹ Stuart, Griffin, Fulton and Anderson, *PROC. SOC. EXP. BIOL. AND MED.*, 1936, **34**, 209.

² Stuart, Griffin, Wheeler and Battey, *PROC. SOC. EXP. BIOL. AND MED.*, 1936, **34**, 212.

³ Weil, *Bioch. Z.*, 1914, **58**, 257.

TABLE I.
Immunization of Guinea Pigs with Raw, Boiled and Autoclaved Beef-Erythrocytes.

Guinea pig No.	Immunized with	No. of injections	Titer for					
			Beef		Sheep		Horse	
			lysins	agglutins	lysins	agglutins	lysins	agglutins
1	Raw beef cells	0	20	0				
		5	320	0	1280	0	0	0
		7	2560	0	640	80	0	80
2	" " "	0	0	0				
		4	640	0	320	0	0	0
		6	640	0	320	320		
		8	1280	80	320	80	0	320
11	Boiled beef cells	0	20	0				
		4	10240	0	320	320	0	
		6	5120	0	320	640	0	640
21	Autoclaved beef cells	0	0	0	0	0		
		2	0	0	0	0		
		5	40	0	0	0		
22	" " "	0	0	0	20	0	0	
		2	1280	0	40	0	0	
		4	5120	0	160	80	80	640
27	" " "	0	40	0	0			
		4	5120	0	80	40	40	40

Table I shows the titers produced by the injection of beef-cells. Guinea pigs 1, 2 and 3 received raw cells, 11 and 12 boiled cells and 21 to 28 autoclaved cells. Normal titers for beef-lysins were 1:20 or lower except in animals 26 and 27. There was considerable variation in the height of the immune beef-lysin titer; boiled and autoclaved cells produced the highest titers.* Only animal 21 failed to produce any significant amount of beef-lysins. Agglutinins for beef-cells were present only in guinea pig 2 in a low titer and then not until after the 8th injection. In contrast to the strong lysins for beef-cells, with the agglutinins low or absent, antibodies for horse-cells were principally agglutinins. Five of the 7 animals forming horse-cell antibodies had agglutinins only while 2 animals immunized with autoclaved beef-erythrocytes produced both lysins and agglutinins. A majority of the animals produced antibodies for sheep-cells. The sheep-lysin titers were considerably lower than the beef except in the serum of animal 1 tested after the 5th injection. Unlike the beef, titers for sheep-cells were higher in animals which received raw cells.

* Heated cells produced severe peritonitis resulting in the death of 8 of the 12 animals injected. This irritation may have served as an added stimulus to antibody formation.

To determine the specificity of the antibodies the immune serums were adsorbed with raw and autoclaved beef- and sheep-erythrocytes and raw horse-erythrocytes. A 1:5 dilution of the serum was adsorbed 3 successive times for one hour each with .25 ml. of packed raw or .5 ml. of packed autoclaved cells for each milliliter of serum-dilution. The titers before adsorption and after the third adsorption are listed in Table II.

TABLE II.
Adsorption of Immune Guinea Pig Serums.

Adsorbed with	Titer for			
	Beef lysins	Sheep lysins	Sheep agglutinins	Horse agglutinins
Anti-raw beef cell serum (aver. 2 serums)				
Control	1280	640	120	320
Raw beef cells	0	0	0	0
Autoclaved beef cells	320	160	0	0
Raw sheep cells	640	0	0	0
Autoclaved sheep cells	480	240	0	0
Raw horse cells	640	640	0	0
Anti-autoclaved beef cell serum (aver. 2 serums)				
Control	7680	160	60	320
Raw beef cells	0	0	0	0
Autoclaved beef cells	0	0	0	0
Raw sheep cells	2560	0	0	0
Autoclaved sheep cells	1920	0	0	0
Raw horse cells	3840	80	0	0

Adsorption with raw beef-cells of serums produced by immunization with raw beef-cells removed all antibodies for beef-, sheep- and horse-erythrocytes.† Raw sheep-cells adsorbed sheep-lysins and sheep- and horse-agglutinins but did not adsorb the beef-lysins. These remaining lysins were presumably antibodies produced by the species-specific isophile beef-cell antigen. Autoclaved beef-, autoclaved sheep- and raw horse-cells removed agglutinins for sheep- and horse-cells but not the sheep- or beef-lysins. The failure of autoclaved sheep-cells to adsorb the sheep-lysins indicated the presence of antibodies for the thermolabile sheep-beef antigen. The removal of sheep-agglutinins with no significant decrease in the lysins appears analogous to the adsorption of serum-sickness serums by beef- and rabbit-erythrocytes. The sheep-cell agglutinins in these serums were completely adsorbed with only a slight reduction in the lytic titer.⁴ These adsorptions indicate that the ag-

† There was one unexplained exception; 5 adsorptions of the serum from animal 2 with raw beef-cells reduced the sheep lysin titer only 75%.

⁴ Stuart, Fulton, Ash and Gregory, in press.

glutinins and lysins were produced by different antigenic constituents of the bovine cell.

Raw and autoclaved beef-cells removed all beef-, sheep- and horse-antibodies from serums produced by immunization with autoclaved beef-erythrocytes. Both raw and autoclaved sheep-cells adsorbed the sheep-lysins. Since the antibodies were produced by injection of autoclaved beef-cells in which no Forssman-antigen has been demonstrated the sheep-lysins in these serums must have been produced by the B.T. antigen. Sheep-cells did not remove the beef-lysins. If these lysins had been produced entirely by the B.T. antigen they should have been adsorbed by sheep-cells and the fact that they were not indicated a second thermostable antigen in the beef-cell. Horse-cells removed the sheep- and horse-agglutinins but did not significantly affect the sheep- or beef-lysins. Furthermore, 3 adsorptions with sheep-cells followed by 3 adsorptions with horse-cells (not shown in the tables) did not remove the beef-lysins. The antigen in the horse-erythrocyte which adsorbed the sheep- and horse-agglutinins, therefore, could not be the same as the B.T. antigen nor identical with the second thermostable antigen in the beef-cell.

Normal rabbit-serum, which contains the B.T. antigen,² was used to inhibit lysis of beef-cells by the immune serums. The technique employed has been previously described.¹ With serums produced by immunization with raw beef-cells inhibition of the beef-lysins was complete in a 1:20 and partial in a 1:40 dilution of the rabbit-serum. Inhibition was somewhat stronger in serums produced by injection of autoclaved cells, being complete in a 1:40 and partial in a 1:80 dilution of the rabbit-serum.

Summary. Adsorptive tests with raw and autoclaved beef- and sheep-erythrocytes, and inhibitive experiments with normal rabbit-serum demonstrated the presence of antibodies for the B.T. antigen in guinea pigs immunized with beef-erythrocytes.

Effect of Hyperthyroidism on Sympathetic Nervous System.

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Much has been written concerning the influence of the sympathetic nervous system upon the thyroid; relatively little on the effect of excessive thyroid secretion upon the sympathetic. The general statement is made that "the thyroid gland appears to sensitize the whole or part of the sympathetic nervous system,"¹ and this is suggested as "the most plausible way of explaining" the retracted upper lids, the exophthalmos and the dilated pupils of exophthalmic goiter.

Most of the experiments reported in the literature indicate that in the state of hyperthyroidism, the sympathetic nervous system is more sensitive to adrenalin. In the course of a series of experiments undertaken for another purpose we have made a number of observations which apparently do not support this view. Nine normal dogs served as controls. Seven dogs were fed 10 gm. of desiccated thyroid daily for from 9 to 14 days immediately preceding the experiments. These animals became very excitable, suffered from diarrhea and lost from 0.61 to 5.0 kg. in weight, or from 3.5 to 32.6% of the original weight.

Under ether anesthesia, cannulae were placed in the trachea and carotid artery and the femoral and jugular veins were exposed, in each animal. The reaction of each dog to one cc. of a fresh 1 to 30,000 solution of adrenalin was determined. The abdomen was then opened and a small rubber tube was placed in position for mechanically constricting the hepatic veins by the method described by Simonds and Brandes.² This tube was left loose so that no constriction of the hepatic veins took place at this time.

In Table I is shown the rise in blood pressure induced by corresponding doses of adrenalin in normal and hyperthyroid dogs.

Table II shows the fall in blood pressure in normal and hyperthyroid dogs induced by merely putting the rubber tube in position for constricting the hepatic veins.

From these experiments it appears (1) that the same dose of adrenalin induces approximately twice as great an average rise in blood pressure in normal dogs as in dogs in a state of chronic

¹ Wright, Samson, *Applied Physiology*, 5th Ed., New York, 1934, p. 155.

² Simonds and Brandes, *Am. J. Physiol.*, 1925, **72**, 201.

TABLE I.
Rise in Blood Pressure from Adrenalin.

Normal Dogs		Hyperthyroid Dogs	
mm. Hg.	%	mm. Hg.	%
24	20.7	16	12.3
23	38.3	25	17.2
40	30.8	7	5.3
10	7.2	20	24.4
23	13.4	7	6.8
34	31.5	12	15.8
20	15.9	6	8.8
39	26.4		
36	30.5		
Aver. 26.6	23.7	13.3	12.9

TABLE II.
Fall in Blood Pressure from Putting Constrictor in Place.

Normal Dogs		Hyperthyroid Dogs	
mm. Hg.	%	mm. Hg.	%
4	3.6	52	45.0
9	10.8	45	32.2
18	11.4	62	47.0
7	5.4	16	20.0
30	16.0	41	38.8
7	6.4	22	29.0
7	5.6	6	8.6
6	4.2		
10	9.1		
Aver. 11.0	8.1	34.9	31.5

hyperthyroidism; and (2) that merely putting a ligature in place for constricting the hepatic veins induces an average fall in blood pressure 3 times as great in hyperthyroid as in normal dogs.

8689 P

Effect of Oestrogenic Hormone Administration upon Nasal Mucous Membrane of the Monkey (*Macaca mulatta*).

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Recently, in this laboratory, an opportunity¹ occurred to investigate the condition of the nasal mucosa in normal monkeys, both

¹ Bachman, C., Collip, J. B., and Selye, H., *Proc. Royal Soc.*, Series B, No. 802, 1935, 117, 16; *Proc. Soc. Exp. Biol. and Med.*, 1936, 33, 549.

mature and immature, and in those treated with crystalline oestrone and oestriol, and with emmenin.

The colony consists of 28 animals. Three are males, 2 immature and one adolescent; 5 are immature females, 4 adolescent females (one a castrate) and 16 are mature females, of which 3 are castrates.

For 8 months nasal examination, using a Cameron speculum, was made weekly; first by 3 examiners, later by 2. The examiners were unaware of the monkey's sex, whether it was mature, and whether it was under treatment. Two other examiners observed nipples, face, back and sex-skin 3 times a week. Their observations were disclosed to the nasal observers after periods of 3 months, when all data were charted on one-year paper.

Normal variations in nasal mucosa color were grouped in a zone extending from "pale" to "healthy red", above which 3 degrees of redness and swelling subsequently came to be recognized. Understatement of impressions of color change was encouraged.

Treated Animals. When nasal examinations commenced there were already 14 animals in course of treatment with oestrogenic hormones; these were studied using the untreated remainder of the colony as controls. Two adult female castrates and one adolescent female castrate, treated with daily hypodermic injection of 500 γ oestrone for 50 to 75 days, were examined during the last 3 to 10 weeks of the period. Typical changes¹ were produced in the nipple, sex-skin, face and back. The 2 adult castrates were examined nasally 12 days after injection had begun, when the conchae showed maximal redness (R.3) in one, and marked redness (R.2) in the other, this becoming maximal in about a week. The peaks were sustained for about 2 weeks; thereafter, despite continued treatment, the color steadily faded till, when treatment was stopped, it had approximately reached normal level, preceding the fading of color in the other sex-skin areas by about a week. The adolescent castrate was seen only during the last 2 weeks of treatment, when the nose showed marked reddening, as did also the nipple, face and sex-skin. Two intact adolescent females, treated similarly, were examined nasally only during the last 2 weeks of treatment. One showed abnormal redness on the 3 occasions examined, while the other did not; this does not mean, of course, that the nose may not have responded earlier in treatment.

Furthermore, nasal reddening was maximal in a young adult female and an immature male treated daily by mouth with one mg. oestriol (theelol) in 10% alcohol, for 70 days, with weekly nasal examination throughout; and a positive nasal response was seen in

9 animals treated with adequate doses of emmenin by oral, hypodermic and intramuscular routes.

Untreated Females. As a control group 12 females were examined under exactly the same conditions as the injected animals. Four were observed for 8 months, 3 for 7 months, 1 for 6 months, 2 for 5½ months, 1 for 4 months and 1 for 3 months; those observed for the shorter periods were added to the series as time went on. At the beginning 7 were cyclic adults, while 5 were described as "immature". Of these last, 3 were adolescent and developed the full cycle, with menstruation, during the latter half of the period of observation. One animal, examined for 4 months, remained immature and acyclic throughout. Two of the cyclic group menstruated irregularly.

Only when the weekly state of the nose was charted on one-year paper together with graphs of redness and/or swelling in the nipple, sex-skin, face and back, together with the indoor and outdoor temperatures and observations as to whether or not the animal had been allowed out, did the following facts become apparent.

1. The nasal variations are not significantly related to temperature variations.

2. There is a seasonal increase in the degree of color of the nasal mucosa as well as in the degree of activity manifest in the several sex-skin areas, with which the former is coincident. In the adults this occurs in September and October, which is the mating season; in the "immature" group, however, it was most marked in November. This may be due to the fact that the series is very small (5 animals), or it may be that the adolescent animal responds somewhat more slowly to the seasonal stimulus.

3. In the untreated cyclic female monkeys there was a periodic rise in nasal mucosa color and activity, most marked in September and October and least in January and February. The peak intervals varied in length in different animals; in the group they averaged 28 days. In the animal in which they were shortest, the average was 21 days, and in the animal in which they were longest 33 days. In the group there was a total of 52 "monkey-months" nasal observation and 45 "nasal peaks" were seen. In 89% of these there was coincident nipple activity, in 84% sex-skin activity, in 69% activity in the face, and in 28% activity in the back (swelling). Finally such nasal peaks were 8% more regular as a manifestation of sexual cyclicity than uterine bleeding. They were found to occur "pre-menstrually" in 60%, coincident with the bleeding in 16% and "post-menstrually" in the remainder. We also observed in

a number of instances that the nasal redness peak in a given month might be double with a remission synchronous with the remission in sex-skin coloration.

In the younger, untreated group 13 nasal peaks were observed in 19 "monkey-months" observation. The intervals were more irregular and somewhat longer than in the adult group, averaging over 30 days; with 77% of them there was coincident nipple activity, in 69% also sex-skin and face activity and in one case there was also swelling of the back. Irregular menstruation or amenorrhea was more frequent than in the adults, which accounts for the fact that in this group the nasal peaks were 16% more regular as a manifestation of sexual cyclicity than uterine bleeding. As regards their position in the cycle more than half of them were clearly "pre-menstrual", while the remainder continued or occurred while bleeding was taking place; none were "post-menstrual". Owing to amenorrhea, only half the peaks could be related to menstrual bleeding.

We should like to make it quite clear that in a cyclic untreated animal, even after months of practice in nasal examination, neither of the observers is of opinion that a single nasal examination would enable him to state with certainty the time in the cycle at which the inspection is made, due to the great variation in color from animal to animal; but they are convinced that observations made over a period of several months will reveal variations that will assume significance when charted with graphs of activity in the other sex-skin areas.

Summary. 1. The nasal mucosa of the intact monkey (*Macaca mulatta*) responds to the administration of oestrogenic substances, crystalline oestrone and oestriol, and emmenin in the manner of and synchronously with the changes known to occur in the nipple, sex-skin, face and back. 2. The response is most clearly seen in the "middle" and inferior conchae which show reddening and/or swelling. 3. The oestrogenic substances mentioned are effective in both females and males, immature and adult animals. 4. The effect can be produced in female castrates by crystalline oestrone and oestriol. 5. In the untreated immature and adult female animal peaks of nasal activity normally occur, chiefly "pre-menstrual" in time, and at intervals of about 28 days. 6. Such naturally occurring nasal cyclical activity is most marked in the autumn months, which is the mating season.

8690 C

Blood Amylase Response to Acetyl-Beta-Methylcholine Chloride in Rabbits.*

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(Introduced by H. Sobotka.)

From the Laboratories of the Mount Sinai Hospital, New York City, and the Newark Beth Israel Hospital, Newark, New Jersey.

In the course of our studies on the blood amylase response to acetyl-beta-methylcholine,^{†1, 2} we attempted to use, for practical reasons, the rabbit as a test animal instead of the dog.

The drug was administered intramuscularly to 13 rabbits, and 24 experiments were performed in this group. The blood amylase was determined prior to and 3 hours after the administration of 4 mg. acetyl-beta-methylcholine chloride. All of the rabbits weighed between 2 and 3 kilos. The fasting blood amylase titre varied from 4.6 to 12.4 units. A marked response was obtained in 18 tests; in 5 there was not so conspicuous a change, and in one no rise was elicited.

The viscosimetric method utilized for these determinations was identical with that reported in the original communication.¹ The findings are summarized in Table I.

It will be noted that in Rabbit 99 a marked blood amylase response was obtained on Sept. 10 and Sept. 16, but that on Sept. 13 the rise was inconspicuous. In Rabbit 13 on Oct. 3 and Oct. 16 the amylase did not reach the level of Oct. 9. In Rabbit 2 there was no response on Oct. 1, while on Oct. 4 more than a 100% increase in the blood amylase was elicited.

As controls saline, 1 mg. histamine, and 5 minims of adrenalin were tested on 6 animals, all of which failed to evoke a rise in the blood amylase titre.

Conclusion. 1. In rabbits, as in dogs, there is a rise in blood amylase following the intramuscular injection of acetyl-beta-methylcholine chloride. 2. The blood amylase response of rabbits to acetyl-beta-methylcholine chloride is not as constant as in dogs.

* Aided by a grant from the Committee on Scientific Research of the American Medical Association.

† Mecholyl-Merek.

¹ Antopol, W., Schifrin, A., and Tuchman, L., *PROC. SOC. EXP. BIOL. AND MED.*, 1934, **32**, 383.

² Tuchman, L., Schifrin, A., and Antopol, W., *ibid.*, 1935, **33**, 142.

TABLE I.

Rabbit	Date	Initial blood amylase units	Blood amylase units 3 hrs. after injection of 4 mg. acetyl-beta-methylcholine chloride
97	9/9	11.0	50
99	9/10	4.6	46
	9/13	6.0	10
	9/16	6.0	30
95	9/19	10.0	82
	9/24	10.0	76
98	9/23	11.0	82
	9/25	8.0	18
3	9/27	12.4	34
	9/30	8.4	100
2	10/1	8.0	8
	10/4	8.8	20
13	10/3	6.0	10
	10/9	7.2	36
	10/16	7.2	12
890	10/8	8.0	150
105	10/15	10.0	50
	10/17	10.0	38
100	10/21	7.6	25
101	10/22	6.2	32
27	2/7	10.0	50
	2/17	8.0	25
6	2/7	10.0	13
	2/17	8.0	28

8691 P

Poliomyelitis Virus and Degeneration of Peripheral Nerves.*

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After intracerebral injection of poliomyelitic virus in *M. rhesus* monkeys, O'Leary, Heinbecker and Bishop¹ showed that motor paralysis preceded noticeable changes in the axones of somatic nerves coming from the segments, the nerves of which supplied the paralyzed muscles. In brief, destruction of nerve cells occurred first, and destruction of nerve fibers secondarily. Since paralysis is produced in from 5 to 8 days following the intracerebral injection of the Flexner strain of virus in *M. rhesus* monkeys, at least that same period of time and perhaps more would elapse before changes would become obvious in the fibers of the somatic nerves, after this method of injection.

Forty-two monkeys were injected subserosally with 25 cc. of a 1% suspension of virus, 10 animals with the same amount and percentage of virus contained in enteric toxin (paratypho-coli filtrate)² subserosally and 7 animals with 0.1 cc. of a 1% suspension of virus intracerebrally. Somatic nerves coming from the lumbar and sacral segments were removed and studied by polarized light between crossed Nicol's prisms.³ Within 24 hours after the injection, the somatic nerves were found to have obvious changes in their fibers, *i. e.*, extensive vacuolization and destruction of fasciculi fibers. In 48 hours, the myelin might be granular, the nodes swollen with spireme-like isotropic threads above and below the cementing discs, and the neurokeratin framework might be prominent around the axis cylinder. The nerve fibers of animals that received virus in enteric toxin were always involved and almost completely destroyed in some sections within 48 hours. The animals that received virus intracerebrally did not show comparable changes,

* Aided in part by a grant received from the President's Birthday Ball Commission on Infantile Paralysis Research.

¹ O'Leary, J. L., Heinbecker, P., and Bishop, G., *Arch. Neur. and Psych.*, 1932, **28**, 272.

² Toomey, John A., *Proc. Soc. Exp. Biol. and Med.*, 1934, **31**, 1015.

³ Sutton, T. S., Setterfield, H. E., and Krauss, W. E., *Bulletin 545, Ohio Agr. Exp. Station*, December, 1934.

even though 10 or more days had elapsed between the time of inoculation and the time the animals were sacrificed.

8692 P

Some Properties of Castle's Intrinsic Factor.

CHARLES FLOOD AND RANDOLPH WEST.

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It has long been known that the activity of pepsin is destroyed by exposure to alkali. Accordingly, fresh normal human gastric juice, collected after histamine injection, was at once brought to pH 10 with NaOH and let stand at room temperature for 30 minutes, after which it was brought to pH 6.0 with HCl. No peptic activity was detected by the hemoglobin method.¹ Using Reimann's technique,² 20 cc. of the juice was mixed with 20 cc. of raw liver hash, incubated at 37°C. and pH 2.5 for 2 hours, then adjusted to pH 5.0, and mixed with boiled tomato juice and fed daily for 7 days to a pernicious anemia patient with red cells at 1,300,000. The reticulocytes rose from 2.8% to a peak of 22.8% on the eighth day, the red cells reaching 2,100,000.

As pepsinogen is not destroyed under the conditions used, a preparation kindly supplied by Dr. John H. Northrop and Dr. R. M. Herriott with a specific activity of 2.3³ and containing 6 mg. total nitrogen per cc. was tested, 4.5 cc. being injected intramuscularly in a case of pernicious anemia, the red cells being at 1,800,000. There was no rise in the reticulocytes nor red cells for the next 10 days, though the patient subsequently had a reticulocyte peak of 29.9% following the oral administration of one dose of 100 gm. boiled liver paste mixed with 100 cc. of normal gastric juice prepared as described for the first case. Boiled liver was used to rule out the effect of hepatic cellular enzymes.

Greenspon's paper⁴ then appeared, suggesting that chilled alkalized gastric juice was effective in pernicious anemia when administered orally 4 hours before meals in doses of 250 cc. daily.

¹ Anson, M. L., and Mirsky, A. E., *J. Gen. Physiol.*, 1932, **16**, 59.

² Reimann, F., and Weil, R., *Z. f. klin. Med.*, 1934, **126**, 568.

³ Holter, H., and Northrop, J. H., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **33**, 73.

⁴ Greenspon, E. A., *J. A. M. A.*, 1936, **106**, 266.

Accordingly, juice was collected by Greenspon's technique, and 80 cc. of juice, incubated at 37°C. for 2 hours, and 80 cc. boiled tomato juice, adjusted to pH 7.0, were administered orally in a single dose to a pernicious anemia patient with red cells at 2,100,000. The reticulocytes were 2.5% and never rose above that level during the next 10 days, the red cells fell to 1,600,000. There was a subsequent rise of reticulocytes to 31% on adequate parenteral liver extract. Another patient with red cells at 1,900,000 received 80 gm. of boiled liver, hashed to a paste, with 80 cc. of boiled tomato juice in a single dose. Reticulocytes rose from 3.3% to a peak of 8.4 on the eighth day, with no change in the red cell level. After the reticulocytes had fallen to 3.3%, 80 gm. of boiled liver paste and 80 cc. of normal juice, collected by Greenspon's technique, were incubated at 37°C. at pH 7.0 for 2 hours, and administered with boiled tomato juice. The reticulocytes rose to 14.6% on day 7 and fell to 8.5% 3 days later when 80 gm. of boiled liver paste and 80 cc. of normal juice collected without alkali, but adjusted to pH 10 for 30 minutes and then incubated with the liver for 2 hours at pH 5.5 were given in tomato juice. The reticulocytes rose to 19.8%, the red cells to 2,100,000 six days later.

Summary. Exposure of normal human gastric juice to pH 10 for 30 minutes destroys peptic activity without materially affecting intrinsic factor. Pepsinogen containing 28 mg. N was inert when administered parenterally. Eighty cc. of normal juice collected by Greenspon's technique was inert but when added to 80 gm. of boiled liver accentuated the hematopoietic effect of the liver.

8693 P

Vasomotor Effects of Blood in Patients with Hypertension.

BEN FRIEDMAN* AND MYRON PRINZMETAL.* (Introduced by
B. S. Oppenheimer.)

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Many investigators have noted increased pressor (or diminished depressor) substances in the blood of patients with hypertension.¹⁻⁴

* Sutro Fellows for Cardiovascular Research.

¹ Major, R. H., *Arch. Int. Med.*, 1927, **40**, 891.

² Lange, F., *Klin. Wchnschr.*, 1933, **12**, 173.

³ Bohn, H., *Ztschr. f. klin. Med.*, 1933, **123**, 558.

⁴ Marx, H., and Hefke, K., *Klin. Wchnschr.*, 1933, **12**, 1318.

Others frequently employing similar methods, have failed to find them.⁵⁻⁹ We have used the denervated rabbit's ear preparation as described by Pissemiski¹⁰ and modified by Katz¹¹ to compare the effect of blood of persons with hypertension and subjects with normal blood pressures. This preparation has been found to be sensitive to known pressor substances in high dilutions (epinephrine 1 to 100,000,000 and pitressin 1 to 150,000), and has an important advantage in that whole undiluted relatively unchanged blood plasma may be used. In the early experiments the inflow pipette was connected to a Mariotte bottle. Subsequently we found a constant volume pump to be more satisfactory.

The perfusion was started with Tyrode's solution and followed with unheated plasma alternating between blood from normal and hypersensitive individuals and noting the changes in pressure with constant inflow. Either citrate or heparin was used as anticoagulant. Generally about one hour elapsed between the time of withdrawal of the blood and the beginning of the experiment.

Eighteen patients with hypertension have been studied. These comprise 8 with benign hypertension, 2 with malignant hypertension, 7 in various stages of glomerulo-nephritis and one with polycystic kidney disease. The effect of the plasma as compared with that of the normal control is summarized in the table.

In 16 cases there was a depressor or no effect and in only 2 instances was there a definite pressor effect of the blood from the subject with hypertension over that from the individual with normal blood pressure. These changes could not be attributed to variations in viscosity or surface tension, since these properties varied only slightly and in no consistent direction. The addition to the test plasma of minute amounts of known pressor substances such as epinephrin and pitressin was followed by a well defined pressor response.

Three patients with acute glomerular nephritis were studied during different phases of their disease. In one instance a depressor effect was observed during the active phase with hypertension and also following recovery. In the other 2 subjects there was evident

⁵ Aitken, R. S., and Wilson, C., *Quart. J. Med.*, 1935, **4**, 179.

⁶ Turriès, J., and Robert, S., *Presse méd.*, 1930, **38**, 85.

⁷ Page, I. H., *J. Exp. Med.*, 1935, **61**, 67.

⁸ Capps, R. B., Ferris, E. B., Taylor, F. H., and Weiss, S., *Arch. Int. Med.*, 1935, **56**, 864.

⁹ De Wesselow, O., and Griffiths, W. J., *Brit. J. Exp. Path.*, 1934, **55**, 45.

¹⁰ Pissemiski, A., *Pflüger's Arch.*, 1914, **156**, 430.

¹¹ Katz, G., *Arch. internat. de pharmacodyn. et de thérap.*, 1934, **49**, 239.

TABLE I.

	Pressor	Unchanged	Depressor
Benign hypertension	1	4	3
Malignant "	0	0	2
Nephritis			
Acute	1	0	3
Subacute or chronic	0	1	2
Polycystic kidney	0	0	1
Total	2	5	11

a depressor effect during the severe active phase when the blood pressure was elevated and a definite pressor effect when the level of the blood pressure declined toward normal level.

This evidence does not favor the existence of a direct peripherally acting circulating pressor substance in hypertension. If, as is likely in renal hypertension, a humoral substance exists which is responsible for the hypertension, it is not like epinephrin, pitressin or other substances having a direct pressor effect, or else not sufficiently pressor so as to be detected by this method.

8694 P

Nature of Peripheral Resistance in Arterial Hypertension.

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The arteriolar hypertonus responsible for hypertension is to be explained by one of the following mechanisms: 1. Increase in sympathetic vasoconstrictor impulses. 2. Circulating pressor substances. 3. A local disturbance in the vessels themselves.

The first factor has been ruled out since anesthetization of vasomotor nerves does not release the vascular hypertonicity.¹

That there is no increase in circulating pressor substances in the blood of patients with hypertension is indicated by the following experiments. Direct cross transfusions were performed between patients with malignant hypertension and subjects with normal cardiovascular systems (these subjects had inoperable carcinomata). Two Unger sets were employed simultaneously so that at no time was there an appreciable change in blood volume in either patient.

* Sutro Fellows for Cardiovascular Research.

¹ Prinzmetal, M., and Wilson, C., *J. Clin. Invest.*, 1936, **15**, 63.

Four such procedures were carried out, the amounts of blood interchanged being 500, 700, 1460 and 2000 cc. respectively. Congo red was injected into one subject before the transfusion. Forty-two percent of the dye was found in the blood of the other subject at the completion of the experiment.

If hypertension is due to a circulating pressor substance, a rise in blood pressure should be expected in the normal subjects with large amounts of hypertensive blood and a fall in pressure in the hypertensive patients with equivalent amounts of normal blood. No such changes were observed in either group.

Experimental hypertension was produced in dogs by the Goldblatt technique.² The tails of such dogs were then perfused alternately with their own heparinized or citrated arterial blood and with blood from normal dogs. A constant volume pump was used and the pressures required to force the blood through the tissues compared. In 4 instances the blood from the hypertensive dogs had a depressor effect and in one case a pressor effect as compared with blood from normal dogs.

Perfusion of denervated normal and hypertensive organs with Tyrode's solution was performed. Ischemia was produced by stopping the perfusion for varying periods of time. It was found that following this procedure the vessels first dilated and then gradually resumed their former tonicity (reactive hyperemia). These observations emphasize the importance of local factors for the maintenance of vascular tonus independent of nervous impulses or of circulating pressor substances.

It is well known that hypertension may occur following disturbances in renal function. Since the arteriolar hypertonus is not neurogenic in origin,¹ it must be mediated through a humoral mechanism. The evidence presented in this and the preceding³ paper indicates that this circulating substance is not directly pressor in itself. It is concluded that in renal hypertension a circulating substance arising directly or indirectly from the kidney, and not directly pressor in itself elicits the pressor response locally in the vessels themselves by some unknown mechanism. Whether the mechanism of essential hypertension is of a similar nature is unknown. The evidence indicates, however, that since the vascular hypertonus is not neurogenic in origin¹ nor due to a circulating substance having a direct pressor effect on the vessels the difficulty must lie apparently in the vessels themselves, although this too may be mediated in some unknown manner through a humoral mechanism.

² Goldblatt, H., *et al.*, *J. Exp. Med.*, 1934, **59**, 347.

³ Friedman, B., and Prinzmetal, M., *Proc. Soc. Exp. Biol. and Med.*, 1936, **34**, 543.

8695 P

Development of Antiviral Properties Within Lymph Nodes.

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Every intradermal injection is intralymphatic and infectious agents introduced by way of a skin wound may reach the lymphatic glands directly.^{1, 2} Specific bacterial agglutinins are formed in the nodes draining the ears of mice into which killed cultures of bacteria have been injected intradermally.³ Experiments now show that the lymph nodes elaborate a principle capable of neutralizing a virus draining to them.

Rabbits were inoculated intradermally in one ear with a standard amount of vaccine virus and in the other with typhoid bacterin. Thirty-two experiments were done. In half of these the ears were amputated after an hour, while in the others the ears were left intact. After later intervals of from 2 hours to 15 days the cervical lymph nodes of both sides, inguinal nodes, bone marrow and spleen were removed and extracts of them were inoculated separately into 3 or 6 normal rabbits (as was serum procured from the same animals at the same time) to test for their content of virus (titration tests). In addition neutralization tests were set up to learn whether these extracts possessed antiviral properties.

Only the extracts of the ears and cervical lymph nodes of the virus-injected side gave rise to virus lesions. A few hours after the injection of standard amounts of virus in the ears equal amounts of virus were recovered from the lymph nodes. By the second or third day an increase in virus was demonstrable in the extracts of the nodes on the virus-injected side. A rapid decline took place thereafter and by the 7th to the 9th days the extracts failed to produce lesions. At no time did the serum or extracts from other nodes or organs give evidence of virus.

Tests showed an early appearance of neutralizing ability in the extracts of the nodes from the virus-injected side. By the 4th day the 10% extracts of these nodes inactivated fresh vaccine virus diluted to 10^{-5} and 10^{-6} (10-100 infective doses) to a greater degree than did whole serum. Serum of the same animal, similarly diluted, possessed no neutralizing power, nor did the extracts of inflamed

¹ Hudack, Stephen S., and McMaster, Philip D., *J. Exp. Med.*, 1933, **57**, 751.

² McMaster, Philip D., and Hudack, Stephen S., *J. Exp. Med.*, 1934, **60**, 479.

³ McMaster, Philip D., and Hudack, Stephen S., *J. Exp. Med.*, 1935, **61**, 783.

cervical lymph nodes from the other side, nor the extracts of spleen or bone marrow or of either ear. The lack of neutralizing power of the extracts of the inflamed nodes on the side injected with typhoid bacterin served to control the possibility that a concentration of antiviral bodies formed elsewhere in the body and circulating in the blood had taken place in the nodes of the virus-injected side. After longer intervals, for example 7 days, the serum and extract of lymph nodes from the virus-injected side, similarly diluted, showed equal neutralizing ability; whole serum possessed even more.

Experiments were done to test whether or not a neutralizing principle might be formed in the tissue of the injected ear, and reach the node by drainage. Extracts of the tissues of both ears (the virus-injected one showing a typical vaccinia lesion) as well as of the cervical nodes of both sides 4 days after inoculation were separately filtered through Seitz pads and tested. The filtration separated virus from antiviral principle in the extract of the cervical nodes from the virus-injected side in which both were present together, only the antiviral properties passing the filters. The ear extracts and filtrates totally lacked antiviral power and so too did those of the lymph nodes on the control side, whereas those of the glands on the injected side possessed it. Virus was present only in the ear extract from the virus-injected side. The experiment was repeated with similar findings.

The results from day to day with serum and extracts of other organs than the lymph nodes indicate that the latter were a major source of the neutralizing power of the serum.

The circumstances of our experiments were much like those when natural infection with a virus takes place by way of cuts or abrasions of skin or mucous membrane. The resemblance to the conditions obtaining in the case of ordinary Jennerian vaccination is especially close. The neutralizing power of the blood is probably of lymph node origin.

8696 C

Fibrinolytic Activity of Hemolytic Streptococci and Other Microorganisms.

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The fibrinolytic activity of hemolytic streptococci has been extensively studied by Tillett and Garner.¹ According to these authors, only hemolytic streptococci, with the exception of some strains of staphylococci, produce fibrinolysin. Cultures of *Streptococcus viridans*, *Streptococcus non-hemolyticus*, enterococcus, pneumococcus, and other microorganisms proved to be ineffective. These findings were recently corroborated by Schmidt.² The object of our investigations was to ascertain the significance of the composition of the culture-medium for the production of the fibrinolysin.

The respective broths (with plain meat-infusion broth, pH 7.4 as a base), were inoculated and incubated at 37°C. for 18 hours. The cultures were centrifuged and the supernatant fluid tested for fibrinolytic activity according to the technique of Tillett and Garner.¹

The plasma used for the experiments was prepared by mixing 10 cc. of human blood with 1 cc. of a 2% potassium oxalate solution. The blood was shaken thoroughly and centrifuged. For the demonstration of the fibrinolysin, the supernatant fluid of the culture, in the amount of 0.5 cc., was mixed with 1 cc. of a 1:5 dilution of human plasma; then 0.25 cc. of a 0.25% calcium chloride solution in normal saline was added, the tubes shaken thoroughly and kept at 37°C. The results were read at various intervals.

The influence of the glucose-concentration of the meat-infusion broth upon the production of the fibrinolytic substance by hemolytic streptococci was first studied. In Experiment I, 3 strains of hemolytic streptococci were incubated for 18 hr. at 37°C. in meat-infusion broth containing 2, 0.4, 0.08, and 0.016% glucose. The supernatant fluid of the respective cultures was tested for fibrinolytic activity.

As can be seen in Table I, strain 693 produced fibrinolysin in 2% and 0.4% glucose broth, but not in 0.08% and 0.016% glucose

* Aided by a Grant of the Emanuel Libman Fellowship Fund.

¹ Tillett, W. S., and Garner, R. L., *J. Exp. Med.*, 1933, **58**, 485; Tillett, W. S., *J. Bact.*, 1935, **29**, 111.

² Schmidt, H., *Z. f. Immunitätsf.*, 1936, **87**, 1.

broth. On the other hand, the formation of fibrinolysin by strain 751 occurred in broth containing glucose from 0.016% to 2%. Strain 646 was completely ineffective. It follows from this experiment that the production of fibrinolysin may depend upon the glucose-concentration of the broth employed, as well as upon the particular strain.

The following experiments dealt with the rôle played by the particular type of carbohydrate of the culture-medium in the production of fibrinolysin. For this purpose, 2 strains of hemolytic streptococci were grown for 18 hr. at 37°C. in (a) plain meat-infusion broth, (b) 2% glucose broth, (c) 2% mannit broth, (d) 2% salicin broth and (e) 2% lactose broth. The cultures were centrifuged and the supernatant fluid tested for fibrinolytic potency. Table II shows that fibrinolysis varies with the type of carbohydrate used in the culture medium, as well as with the reading time. It is evident that glucose can be replaced by other carbohydrates. The fibrinolysin produced in some of the sugar-broths inhibits coagulation of the plasma continually; that of other carbohydrate-broths, however, is effective only temporarily inasmuch as fibrinolysis precedes or follows coagulation of the plasma. There seems to be no definite relationship between the growth of the respective strains and their fibrinolysin-production in the various sugar-broths. On the other hand, it was found that some strains of hemolytic streptococci produced the fibrinolytic substance after 48 but not after 18 hr. in the same medium. Further investigations will ascertain whether a parallelism exists between the production of fibrinolysin in the various sugar-broths and the fermentation of these carbohydrates.

The production of fibrinolysin has been described as a specific property of hemolytic streptococci. Since the above experiments showed the significance of the medium for the production of the fibrinolytic substance, the question of specificity was reconsidered. Various types of microörganisms in 2% glucose broth, were tested for fibrinolytic activity. Thirty-one of 40 strains of hemolytic streptococci produced fibrinolysin. Of 47 strains of *Streptococcus viridans*, 35 were found to be fibrinolytic. This result corroborates the observations of Tunncliff,³ who very recently reported fibrinolysin production by several strains of *Streptococcus viridans*. Thus far, 31 strains of enterococcus (streptococcus fermenting æsculin) were tested and all found to be fibrinolytic. Of 78 strains of pneumococci, 31 strains produced fibrinolysin, while 47 strains did not.

³ Tunncliff, R., *J. Inf. Dis.*, 1936, **58**, 92.

The distribution of fibrinolytic and non-fibrinolytic strains of pneumococci was about the same among all the types tested.

Preliminary studies with various kinds of bacilli such as *B. coli*, *B. lactis aerogenes*, *B. friedländeri*, *B. pyocyaneus* and *B. proteus*, revealed that several strains of these microorganisms were able to produce fibrinolysin when cultured in meat-infusion broth containing 2% glucose.

It may be mentioned that sometimes it was possible to differentiate various strains of *Streptococcus viridans* obtained from one specimen of sputum by means of their fibrinolytic potency. Some of the colonies proved to be fibrinolytic, while others were not, when cultivated under identical conditions in 2% glucose meat-infusion broth.

Further experiments were concerned with the question whether enterococci, pneumococci and other microorganisms produce a fibrinolysin if the glucose of the medium is substituted by other carbohydrates, such as lactose, saccharose, maltose, mannit, galactose and xylose. As in the case of hemolytic streptococci, the production of the fibrinolysin varied with the particular carbohydrate and the strain.

It follows from our experiments that various types of microorganisms are capable of producing fibrinolysin. These findings seem to contradict the observations of Tillett and Garner that the production of fibrinolysin is specific for hemolytic streptococci. The following experiment may offer an explanation for the differences in the results obtained: One strain each of *Streptococcus hemolyticus*, *Streptococcus viridans* and enterococcus, was grown in (a) 2% glucose-broth, (b) 0.05% glucose-broth, for 18 hr. at 37°C. The supernatant fluid of the respective cultures was examined for fibrinolytic potency. Fibrinolysin was produced by *Streptococcus hemolyticus* in 0.05% glucose-broth, as well as in 2% glucose-broth. On the other hand, *Streptococcus viridans* and enterococcus did not show any fibrinolytic activity when cultured in 0.05% glucose-broth, but produced the fibrinolytic substance in 2% glucose-broth. The results of Tillett and Garner are corroborated in that only hemolytic streptococci produce the fibrinolysin in 0.05% glucose-broth. However, the fibrinolysin-production is not limited to hemolytic streptococci alone, if, for instance, the sugar-content of the culture-medium is increased.

8697 C

Detoxification of Dendrobine by 'Sodium Amytal'.

K. K. CHEN AND CHARLES L. ROSE.

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Work in this laboratory^{1, 2} has demonstrated that 'Sodium Amytal' (Sodium Iso-amyl Ethyl Barbiturate, Lilly) can save rabbits poisoned with 35 minimal lethal doses of strychnine sulphate, 40 minimal lethal doses of picrotoxin, and 45 minimal lethal doses of coriamyrtin. In cocaine poisoning, only 4 minimal lethal doses can be antidoted by the same hypnotic, and this requires almost simultaneous administration of the convulsant subcutaneously and the barbiturate intravenously.³ In view of the fact that dendrobine, an alkaloid of the Chinese drug Chin-shih-hu (a species of *Dendrobium*),⁴ causes convulsions of central origin,⁵ it was thought interesting to find out whether or not there was any antagonism between dendrobine and 'Sodium Amytal'.

Our preliminary experiments were made in mice by mixing and injecting subcutaneously the calculated doses of dendrobine hydrochloride and 'Sodium Amytal'. One per cent solution of each was employed. As shown in Table I, the minimal lethal dose of dendrobine HCl by subcutaneous injection is 120 mg. per kg., as contrasted with 20 mg. by vein.⁵ By the addition of 'Sodium Amytal', at least 2½ minimal lethal doses of dendrobine HCl (300 mg. per

TABLE I.
Detoxification of Dendrobine by 'Sodium Amytal' in Mice by Subcutaneous Injection.

Dendrobine HCl	'Sodium Amytal'	No. of Mice Used	No. of Mice Died
mg. per kg.	mg. per kg.		
110	0	5	0
120	0	4	4
150	0	1	1
120	75	5	2
180	75	3	0
240	75	5	5
240	100	5	1
300	100	8	3
360	100	5	3
360	125	5	5

¹ Swanson, E. E., *J. Lab. and Clin. Med.*, 1933, **18**, 933.

² Swanson, E. E., and Chen, K. K., *J. Pharm. and Exp. Therap.* (in press).

³ Swanson, E. E., *J. Lab. and Clin. Med.*, 1932, **17**, 325.

⁴ Chen, K. K., and Chen, A. L., *J. Biol. Chem.*, 1935, **111**, 653.

⁵ Chen, K. K., and Chen, A. L., *J. Pharm. and Exp. Therap.*, 1935, **55**, 319.

kg.) were detoxified. Apparently, the optimal dose of 'Sodium Amytal' in the detoxification of dendrobine in mice, in the manner described, is 100 mg. per kg.

The next series of experiments were carried out in rabbits (gray chinchilla) by giving dendrobine HCl subcutaneously. Upon the appearance of the first convulsion, 'Sodium Amytal' was injected intravenously, and repeated when convulsions reappeared. The strength of solution of each substance was 10% in water. The initial dose of the barbiturate was 25 mg. per kg. ($\frac{1}{2}$ of the minimal anesthetic dose), and all subsequent doses were reduced to 12.5 mg. per kg. ($\frac{1}{4}$ of the minimal anesthetic dose). From Table II, it may be seen that the subcutaneous minimal lethal dose of dendrobine HCl in rabbits is 100 mg. per kg., as compared with 17 mg. by intravenous injection.⁵ By repeated administration of 'Sodium Amytal,' convulsions ceased, rapid breathing diminished, and 3 out of 4 animals recovered from 500 mg. of dendrobine HCl per kg. of body weight (5 minimal lethal doses). The remaining rabbits, except one, all died from doses amounting to 600 or more mg. per kg., in spite of treatment.

TABLE II.
Detoxification of Dendrobine by 'Sodium Amytal' in Rabbits.

Rabbit No.	Sex	Body Wt. kg.	Dendrobine HCl (Subcutaneous) mg. per kg.	'Sodium Amytal' (Intravenous) mg. per kg.	Result
1	—*	2.355	90	0	survived
2	—*	2.200	90	0	"
3	—*	2.730	90	0	died
4	—*	2.071	90	0	"
5	—*	1.584	90	0	survived
6	—*	1.800	100	0	died
7	—*	2.340	100	0	"
8	—*	2.570	100	0	"
9	M	1.350	200	50.0 in 3 doses	survived
10	F	1.847	400	62.5 " 4 "	"
11	M	1.500	500	25.0 " 1 "	died
12	F	1.640	500	50.0 " 3 "	survived
13	F	1.670	500	75.0 " 5 "	"
14	F	1.641	500	75.0 " 5 "	"
15	F	1.300	600	50.0 " 3 "	"
16	M	1.634	600	62.5 " 4 "	died
17	F	1.416	600	50.0 " 3 "	"
18	F	1.740	600	37.5 " 2 "	"
19	M	1.435	700	62.5 " 4 "	"
20	F	1.602	800	50.0 " 3 "	"
21	F	1.528	1000	37.5 " 3 "	"

*Not recorded.

Summary. 'Sodium Amytal' detoxifies dendrobine. In rabbits it can antidote 5 minimal lethal doses of the alkaloid after definite signs of intoxication have been observed.

Toxin-Antitoxin Flocculation in Tetanus.

S. EDWARD SULKIN. (Introduced by J. Bronfenbrenner.)

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While the flocculation-test with diphtheria-toxin and antitoxin has given fairly good results,^{1, 2} the method has been found unreliable when applied to toxins and respective antitoxins of tetanus,^{3, 4, 5} botulinus,^{6, 7} staphylococcus,⁸ and *Streptococcus scarlatinae*.^{9, 10}

Bronfenbrenner and Reichert⁶ found that antitoxin obtained by immunizing animals against toxic filtrates of 24-day cultures of *B. botulinus* precipitated the filtrate while antitoxin prepared against toxic filtrates of 4-day cultures did not precipitate. They concluded that precipitation in the Ramon test may be influenced by the antibacterial antibody in the antitoxic serum. It seemed interesting to see whether these findings might also account for the discrepancies reported^{3, 4, 5} in the *in vitro* titration of tetanus-toxin and antitoxin.

Rabbits were immunized with the formolized filtrates of *B. tetani* grown in 1% dextrose-ground beef-broth for 4 and 20 days, respectively, as well as with filtrates of 4-day and 20-day cultures of an atoxic variant of the same strain of *B. tetani*. As expected, the antisera prepared against the nontoxic filtrates were found to be entirely devoid of antitoxin as determined by the protection-test on mice, since as much as 0.5 cc. of undiluted serum failed to protect mice against even one m.l.d. of toxin. The sera containing antitoxin and those obtained against the nontoxic filtrates were used in the precipitation-test with the following antigens: (1) filtrates of 4-day cultures of the toxic and atoxic variants; (2) filtrates of 20-day cultures of the toxic and atoxic variants; and (3) 24-day filtrates of Type "A" botulinus-toxin (control). At the same time *in vivo* neutralization-tests were also carried out, and in the table

¹ Bayne-Jones, S., *J. Immun.*, 1924, **9**, 481.

² Povitzky, O. R., and Banzhaf, E. J., *PROC. SOC. EXP. BIOL. AND MED.*, 1924, **22**, 11.

³ Kalie, D. Z., *Compt. Rend. Soc. Biol.*, 1928, **98**, 649.

⁴ Inoue, T., *Bull. l'Inst. Past.*, 1932, **30**, 246.

⁵ Schmidt, S., *Ann. l'Inst. Past.*, 1928, **42**, 63.

⁶ Bronfenbrenner, J., and Reichert, P., *J. Exp. Med.*, 1926, **44**, 553.

⁷ Tani, S., *Japan. J. Exp. Med.*, 1934, **12**, 33.

⁸ Sulkin, S. E. (to be published).

⁹ Eagles, G. H., *Brit. J. Exp. Path.*, 1927, **8**, 403.

¹⁰ O'Brien, R. A., Okell, C. C., Birkhaug, K. E., *Brit. Med. J.*, 1926, **2**, 513.

TABLE I.

		Antigen (2.0 cc. in each tube).*				Control
		Filtrate of 4-day culture of toxic strain (10,000 mld per cc.)	Filtrate of 4-day culture of atoxic variant	Filtrate of 20-day culture of toxic strain (20,000 mld per cc.)	Filtrate of 20-day culture of atoxic variant	24 day filtrate Type "A," botulinus toxin (2000 mld per cc.)
cc.						
Antiserum against 20-day filtrate of toxic variant	.08	—	—	+	—	—
	.06	—	—	+	++	—
	.05	—	—	++	+++	—
	.04	—	—	+++	+++	—
	.03	—	—	+++ (N)	+++	—
	.025	—	—	+++	+++	—
	.02	— (N)	—	+++	+++	—
	.01	—	—	+++	+++	—
Antiserum against 20-day filtrate of atoxic variant	.008	—	—	+	—	—
	.005	—	—	—	—	—
	.09	—	—	—	+	—
	.07	—	—	+	++	—
	.05	—	—	++	+++	—
	.03	—	—	+++	+++	—
	.01	—	—	+++	+++	—
	.009	—	—	+++	+++	—
	.007	—	—	+++	+++	—
	.005	—	—	—	—	—

(N) = neutral point in protection test.

++++ = copious precipitation.

+++ = moderate precipitation.

++ = slight precipitation.

+ = no precipitation.

— = no precipitation.

Note: Antisera against 4-day toxic as well as that against 4-day atoxic filtrates failed to precipitate any of the antigens and therefore were omitted from this table.

the letter "N" indicates the neutral point as determined by this method. A wide zone of precipitation occurred when the antitoxic serum prepared against the 20-day formolized filtrate was mixed with either its homologous toxic filtrate or with the 20-day filtrate of the atoxic variant. The antiserum prepared against the 20-day filtrate of the atoxic variant also produced marked precipitation in the presence of both the homologous filtrate and of the 20-day filtrate of the toxic strain. On the other hand, in the case of the antitoxin obtained against the 4-day filtrate of the toxic variant no precipitation occurred in the presence of the various filtrates tested, although this serum neutralized the toxin in the *in vivo* tests. Similarly, no precipitation occurred when the antiserum prepared against the 4-day filtrate of the atoxic variant was combined with the respective antigens.

The results of these experiments indicate that (1) the filtrates of the old cultures of the toxic variant contain bacterial protein in addition to the toxin, thereby stimulating the production of both antibacterial antibodies and antitoxin; (2) that the filtrates of the young cultures of the toxic variant are relatively free from bacterial protein and hence their antisera contain antitoxin and no detectable antibacterial antibody; (3) that precipitation with tetanus-toxin and antitoxin does not result primarily from the union of toxin and antitoxin.

Similar experiments with staphylococcal toxin and antitoxin will be reported subsequently.

8699 C

Effect of Dinitrophenol on Agglutinin and Complement Titer.

A. A. DAY AND RUTH W. JUNG.

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The prompt and striking pharmacologic actions, sustained increases in metabolism and body-temperature, produced in man and animals by dinitrophenol, suggested its employment to test the influence of these altered functions on immunologic reactions. Rabbits 1, 2, 3 and 4 were given daily or twice daily, subcutaneous injections of 10 mg. gradually increasing to 24 mg. of dinitrophenol (2.4) mg./k. body-weight over a period of 37 days. The solution was made up in 3% volume with $\frac{1}{2}$ weight of NaHCO_3 . On the

first, fourth, eighth and eleventh days of the dinitrophenol administration the rabbits and 2 control rabbits, 5 and 6, each received a subcutaneous injection of one cc. of a heat-killed suspension of *B. paratyphosus* B. for the production of agglutinins. The animals gained weight and evidenced no apparent effect of the drug other than a rise of 2 to 3 degrees in temperature. Quantitative agglutination-tests failed to show any influence of the dinitrophenol on the rate or extent of production of agglutinins (Table I).

TABLE I.
Agglutinin Titer.

Days	Test Rabbits (DNP)					Control Rabbits (No DNP)		
	1	2	3	4	Av.	5	6	Av.
0	0	0	0	0	0	0	0	0
7	600	640	640	1280	800	2560	640	1600
14	1600	1600	1600	1600	1600	3200	3200	3200
16	800	800	1600	6400	2400	3200	3200	3200
18	1600	1600	6400	6400	4000	3200	—	3200
22	1600	—	3200	1600	2100	—	3200	3200
28	800	—	3200	800	1600	1600	1600	1600
37	800	800	—	800	800	—	400	400

At the end of the administration-period the animals were given a single larger dose of dinitrophenol. Rabbit 1 received 35 mg./k., the temperature rose to 105.6°F. and no change in agglutinin-titer occurred. Rabbit 2 was given 35 mg. per kilo, the temperature increased to 106.3°F. with no change in titer. Rabbit 3 survived the injection of 40 mg./k. with a rise in temperature of 4.1°F. and no significant alteration in titer. Rabbit 4, given 35 mg./k., responded with an increase in temperature from 102.2 to 105.4°F., with no change in titer.

Five of the animals were allowed to rest until the agglutinin-titer declined, from 4 to 11 months, and were then given another large dose of dinitrophenol to see whether a secondary advance in titer would result. Temperatures of from 105° to 110°F. were attained. The agglutinin-titer was unchanged except in one rabbit in which it increased from 1:600 to 1:2000.

The effect of dinitrophenol on the complement-content of rabbits 1, 2 and 6 was also determined. The complement-titer of rabbit 1 was determined before and after the administration of 30 mg./k. of dinitrophenol, followed by 14 mg. per kilo, when the temperature had reached 106°F. Table II shows a very slight decrease in complement at 107.6°F. The test was repeated, using the native hemolytic amboceptor present in the rabbit-serum. Here

the difference was more marked. To ascertain whether the change was in the complement or native hemolysin the serum was inactivated and guinea pig's complement added. Here, the uniform titers indicate that the change observed after the injections of dinitrophenol was in the complement (Table II).

Rabbits 2 and 6, following the injection of 30 mg./k., exhibited no essential change in complement titer.

Tests of uninjected rabbit's serum revealed no significant diurnal variation in complement or hemolysin.

Summary. Subcutaneous administration of dinitrophenol in varying amounts from 10 mg. to 24 mg./k., over a period of 37 days, had no apparent effect on the production of agglutinins for *B. paratyphosus* B in rabbits. Large amounts, 30 to 40 mg. per kilo, sufficient to increase the temperature several degrees, did not significantly alter the agglutinin-titers. Similar large doses given after a rest period of from 4 to 11 months failed to cause a secondary rise in titer except in one instance. A very slight decrease in complement-content took place in 2 of 3 experiments following large doses of dinitrophenol. The above results did not encourage more extensive investigation.

8700 P

Vasomotor Response of Non-Hypertensive Individuals to a Standard Cold Stimulus.

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Hines and Brown¹ have described a standard test for the study of vasomotor reactions based on the responses of the blood pressure to a cold stimulus. They are of the opinion that "excessive" responses to this test indicate a hypersensitive sympathetic nervous system. Assuming that the principal abnormality in essential hypertension is a hypersensitive sympathetic nervous mechanism, they have considered non-hypertensive individuals with "excessive" responses potential candidates for hypertension. They suggest that essential hypertension develops only in individuals manifesting "ex-

¹ Hines, E. A., Jr., and Brown, G. E., *Ann. Int. Med.*, 1933, 7, 209.

cessive" responses; that these abnormal responses depend upon an hereditary factor, and that they appear early in life. If these theories are correct, it is apparent that in advanced age periods where actual essential hypertension has already developed in many of the potential hypertensives, there should be a lower percentage of "excessive" responses in the individuals of the same age period with normal blood pressures. Also, it would follow that the average response of non-hypertensive people in the advanced age periods would be lower than the average response of non-hypertensives in younger age groups.

It is the purpose of this study to investigate the incidence of "excessive" responses in a group of non-hypertensive subjects; and, to compare the average response of this group with the series reported by Hines and Brown.²

Two hundred and five subjects with a systolic blood pressure below 140 mm. Hg. and a diastolic blood pressure below 90 mm. Hg. have been chosen at random. The ages of the individuals selected have varied from 8 to 70. Each subject has been tested according to the technic described by Hines and Brown.¹

The upper limit of normal response was first arbitrarily placed by Hines and Brown¹ at 15 mm. Hg., but in a subsequent publication² they raised their limit of normal to 22 mm. Hg. For present purposes 15 and 22 mm. Hg. have been considered separately as the upper limit of normal. Then, percentages of abnormal responses have been grouped according to age. The results of this analysis are presented in Table I. It will be seen that in no instance is there an appreciable decrease in the percentage of abnormal responses in the older age groups.

TABLE I.

Age	Below 30	30 and over	Below 40	40 and over	Below 50	50 and over
No.	135	70	171	34	192	13
Systolic over 15 mm. Hg. (%)	46.6	55.7	51.4	41.2	48.9	53.8
Systolic over 22 mm. Hg. (%)	14.8	24.3	18.1	17.6	18.2	15.4

In Table II the average responses of our series and that of Hines and Brown² are grouped according to age periods. It will be observed that the 2 series, comprising more than 500 normal individuals, are in close accord. It will be noted further that the aver-

² Hines, E. A., Jr., and Brown, G. E., *Am. Ht. J.*, 1936, **11**, 1.

TABLE II.

Age in years	No.	Systolic mm. Hg.	Diastolic mm. Hg.
6-19	30	14.2	13.4
20-39	141	16.4	16.2
40-	34	15.2	13.1
	(From Hines and Brown)		
0-20	89	13.4	12.3
20-40	201	12.9	11.8
40-	98	19.6	18.4

age response to cold in patients 40 or more years of age in the series of Hines and Brown is greater than the average of their younger age groups, and that there is no significant difference in the present series.

It must be emphasized that the number of reported cases of non-hypertensive individuals studied with the cold water test is too small for any conclusion. The findings of the present study indicate that non-hypertensive older persons are as likely to give "excessive" responses to the test as non-hypertensive younger persons. This suggests that as many of the younger subjects with normal responses may develop hypertension as those with "excessive" responses. If this be true the original contentions of Hines and Brown would be invalidated.

8701 P

Limitation of Fluorine Toxicosis in the Rat with Aluminum Chloride.*

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From Henry Ford Hospital, Detroit.

Fluorine fed in low concentration has been shown to cause defective teeth in guinea pigs, rats, dogs, and cattle. In higher concentrations it causes inferior reproduction, poor bones and inferior growth or death.¹ Recently it has been shown that mottled enamel of human teeth is caused by the toxic action of fluorides present in drinking water.²

* Read before the American Institute of Nutrition, Washington, D. C., March 25, 1936.

¹ McClure, F. J., *Physiol. Rev.*, 1933, **13**, 277.

² Smith, M. C., Lantz, E. L., and Smith, H. V., *Arizona Agr. Exp. Sta. Tech. Bull.*, 1931, **32**.

This is a report of some work done in the search for a substance that would limit the toxic action of fluorine. Salts of lanthanum, cerium, thorium, and boron, which form insoluble or complex fluorides were found to give either no protection or, as in the case of lanthanum, only slight protection. Aluminum chloride was found to inhibit to a large extent the toxic effect of fluorine.

The basal diet used was as follows: yellow corn meal 30.0 parts, ground whole wheat 30.0 parts, whole milk powder 30.0 parts, linseed meal 5.0 parts, alfalfa meal 3.0 parts, and wheat germ 1.0 part.

Fluorine was added to this diet as a solution of sodium fluoride. Aluminum chloride was also added in solution. When both were added the solutions were combined.

Sodium fluoride was fed in 2 concentrations, 0.025 and 0.1% of the diet. Aluminum chloride was fed in concentrations of 0.5% and 2.0%, which is equivalent to 0.056 and 0.224% aluminum respectively.

Effect upon Growth. Sodium fluoride fed at a level of 0.025% of the diet had no effect on the rate of growth. Animals fed the diet containing 0.1% sodium fluoride grew at approximately two-thirds the normal rate. By adding 0.056% aluminum to the diet as the chloride, normal growth was obtained.

Since it has been shown that calcium fluoride is less toxic to growth than sodium fluoride³ the effect of calcium salts added in place of the aluminum was also studied. Calcium was added as calcium carbonate, and as calcium chloride. One percent calcium carbonate gave some protection and 2% caused normal growth. The addition of 1% calcium chloride caused a lower rate of growth than that obtained from sodium fluoride alone.

Effect upon Incisors. The most delicate evidence of fluorine toxicosis in rats is its effect upon the incisors.³ The surface of the incisors of rats fed a diet containing 0.025% sodium fluoride appeared dull and white, the lowers had a tendency to wear excessively, and allow the uppers to overgrow. The incisors of rats fed the same diet containing 0.5% aluminum chloride appeared normal for 5 to 6 weeks, when the uppers began to bleach and the lowers showed orange and white bands which could be distinguished with the naked eye. With 2.0% aluminum chloride they appeared normal to the naked eye (Fig. 1) (observed 6 mos.), but when examined with a hand lens, fine alternate dark and light pigment lines could be seen.

³ Smith, M. C., and Leverton, R. M., *Ind. and Eng. Chem.*, 1934, **26**, 791.

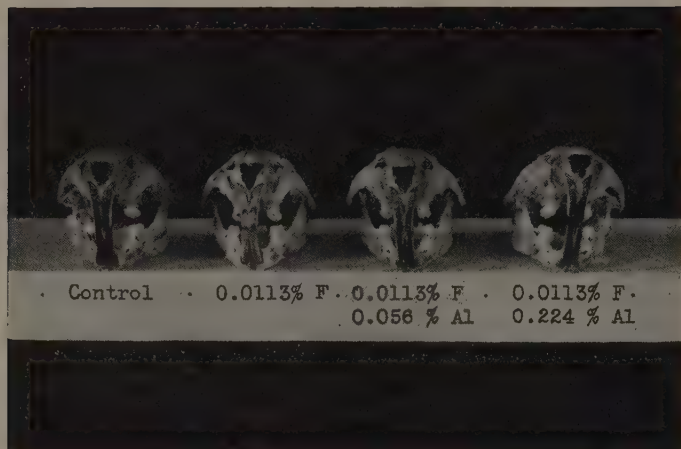


FIG. 1.

Showing toxic effect of fluorine on incisors and prevention by aluminum.

Rats fed the diet containing 0.1% sodium fluoride had incisors with a rough and often pitted surface, and sections of the enamel were often chipped off. Rats fed this diet with 0.5% aluminum chloride had incisors with a smooth surface but they were bleached and the lowers wore down rapidly. Two percent aluminum chloride produced incisors with a smooth pale yellow surface which sometimes showed broad white and yellow bands. Two percent calcium carbonate gave no significant protection to the incisors. However, 2.0% calcium carbonate and 2.0% aluminum chloride produced incisors which appeared nearly normal to the naked eye. With a hand lens fine orange and white bands could be seen.

Since aluminum is not absorbed by the rat, it is assumed that this protective action must take place in the intestinal tract, probably by the formation of an aluminum and fluorine containing compound which is very slightly dissociated.

Experiments are in progress using smaller concentrations of fluorine and also studies using other aluminum salts.†

† Dr. E. K. Nelson, Iowa State College, Ames, Iowa, has obtained similar results with aluminum sulphate. (Private communication.)

8702 P

Antagonistic Action of Dinitrothymol and Dinitrocresol on Cellular Respiration.

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In an extensive study of the biological effects of nitro and dinitrophenols¹ it was found that extremely small concentrations of 2,4-dinitrothymol (1-hydroxy, 3-methyl, 6-isopropyl, 2,4-dinitrobenzene) blocked the cell division of fertilized sea urchin (*Arbacia punctulata*) eggs without, in any concentration, giving more than a slight stimulation of oxygen consumption. This behavior was in marked contrast to that of certain other nitro compounds, including 4,6-dinitrocarvacrol, the isomer of 2,4-dinitrothymol, which displayed a stimulating action on respiration, increasing to an optimum and then progressively disappearing as the concentration of reagent was made larger. These nitro compounds which stimulated respiration also reversibly blocked the cell division of fertilized sea urchin eggs, the initial concentration for division block being just equal to or slightly larger than that producing the optimum respiratory rise.

The results with dinitrothymol suggest that the above outlined effects of 4,6-dinitro-o-cresol and other nitrophenol derivatives on respiration may conceivably be resolved into two factors. According to this view, one factor leads to stimulation, the other factor to limitation of the rate of oxygen consumption. The extent to which oxygen consumption can be made to rise above normal will then depend on the rate at which the stimulating and limiting factors change with respect to each other as the concentration of reagent is varied.

According to this hypothesis, dinitrothymol shows only a small stimulating effect because its ability to set a top limit to the extent of respiratory rise becomes evident at concentrations which might, if no limiting factor entered, lead to stimulation. That such a limiting factor may play a rôle is indicated by the representative data in Tables I and II.

Measurements of oxygen consumption and the extent of cell division were made with fertilized eggs of the sea urchin (*Arbacia punctulata*) using a technique described elsewhere.¹

Table I shows that 10^{-6} molar dinitrothymol can entirely nullify

¹ Clowes, G. H. A., and Krahle, M. E., *J. Gen. Physiol.*, in press.

TABLE I.

Concentration 2,4-dinitrothymol moles per liter	Concentration 4,6-dinitro-o-cresol moles per liter	Oxygen Consumption c.mm. O_2 per 10 c.mm. eggs	Divisions per egg in 3 hours
0	0	2.76	4.00
0	10^{-6}	3.70	4.00
0	5×10^{-6}	8.25	1.86
0	10^{-5}	8.70	0.39
10^{-6}	0	2.44	1.80
10^{-6}	10^{-6}	2.60	1.49
10^{-6}	5×10^{-6}	2.50	0.14
10^{-6}	10^{-5}	2.65	0

TABLE II.

Concentration 2,4-dinitrothymol moles per liter	Concentration 4,6-dinitro-o-cresol moles per liter	Oxygen Consumption c.mm. O_2 per 10 c.mm. eggs	Divisions per egg in 3 hr.
0	0	2.65	3.80
10^{-7}	0	2.72	3.80
2×10^{-7}	0	2.68	3.84
4×10^{-7}	0	2.76	3.82
8×10^{-7}	0	2.76	3.59
1.6×10^{-6}	0	2.18	0.84
0	4×10^{-6}	7.02	3.68
10^{-7}	4×10^{-6}	6.65	3.64
2×10^{-7}	4×10^{-6}	5.75	2.52
4×10^{-7}	4×10^{-6}	4.03	1.06
8×10^{-7}	4×10^{-6}	3.16	0
1.6×10^{-6}	4×10^{-6}	2.36	0

the stimulating effect of an equal, 5 times greater, or 10 times greater concentration of dinitroresol.

Table II illustrates the effect of varying concentrations of dinitrothymol combined with a single concentration of dinitroresol. With increasing dinitrothymol concentrations, which alone have little or no respiratory effect, the respiratory rate in the presence of the combined reagents falls from an elevated one, produced by dinitroresol alone, to a point approximating the normal value. Beyond this level, a large increase in dinitrothymol concentration is required to produce any further significant decrease in respiratory rate.

The division suppressing effects of the two reagents are additive.

8703 P

**Influence of Temperature on Stimulation of Oxidation
By Dinitro, Dihalo and Trihalophenols.**

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Nitro and dinitrophenols have been shown to stimulate the metabolic processes of many types of cells^{1, 2} and to produce a reversible block to the cell division of fertilized eggs of a number of marine invertebrates. The analogous dihalo and trihalophenols exhibit similar properties when tested on marine eggs, but are unable to cause metabolic stimulation in intact mammals and other animals.³

Various tissues were subjected to treatment with 4,6-dinitro-o-cresol and a number of dihalo and trihalophenols at a series of environmental temperatures, oxygen consumption of the tissues being measured with Warburg microrespirometers.⁴ The tissues included: fertilized eggs of *Arbacia punctulata* and *Nereis limbata*; a commercial baker's yeast (Anheuser-Busch), and various rat tissues.

From the data in Table I concerning the effect of various concentrations of 4,6-dinitro-o-cresol on *Arbacia* at 12°C., 20°C., and

TABLE I.
Stimulation of Oxygen Consumption of Fertilized Eggs of *Arbacia* and *Nereis* by 4,6-dinitro-o-cresol at Various Temperatures. Eggs and Reagent in Sea Water at pH 8.2.

Concentration 4,6-dinitro-o-cresol moles per liter	c.mm.O ₂ per hour per 10 c.mm. of eggs					
	Arbacia			Nereis		
	12°	20°	27°	13°	20°	28°
0	1.67	3.22	5.55	0.63	1.47	2.22
10 ⁻⁶	2.18	3.92	6.95	0.84	2.08	2.81
2x10 ⁻⁶	2.74	6.37	9.10	1.11	2.28	3.07
4x10 ⁻⁶	4.36	9.66	11.80	1.31	2.68	3.13
8x10 ⁻⁶	6.10	9.42	11.05	1.91	4.02	4.12
1.6x10 ⁻⁵	5.90	8.42	10.35	2.82	5.80	5.95
3.2x10 ⁻⁵	5.20	7.20	9.40	3.74	7.75	—
6.4x10 ⁻⁵	4.17	5.33	7.70	4.09	7.20	9.90
1.28x10 ⁻⁴	3.06	3.89	6.10	3.89	6.55	9.95
2.56x10 ⁻⁴	2.83	3.08	4.70	3.02	5.25	7.80
5.12x10 ⁻⁴	2.36	2.33	3.68	2.97	5.15	7.05
1.02x10 ⁻³				2.19	3.87	5.90

1 Clowes, G. H. A., and Krah1, M. E., *Science*, 1934, **80**, 384.

2 Clowes, G. H. A., Kelteh, A. K., and Krah1, M. E., *Biol. Bull.*, 1935, **69**, 341.

3 Krah1, M. E., and Clowes, G. H. A., *Proc. Soc. Exp. Biol. and Med.*, 1935, **33**, 477.

4 Dixon, M., *Manometric Methods*, 1st Edition, Cambridge, 1934.

27°C., and on Nereis at 13°C., 20°C., and 28°C., it may be seen in each case that the optimum concentration for oxidative stimulation does not vary greatly with temperature. At the respective optimum concentrations, the number of units of excess oxygen taken up by the treated eggs increased as the temperature was raised. However, because of the greater rate of increase in oxygen consumption by the control eggs, the percentage stimulation decreased with increasing temperature.

TABLE II.
Stimulation of Oxygen Consumption of Yeast by Dinitroresol, Dichlorophenol, and Trichlorophenol at Various Temperatures. The Suspension Medium Was 0.05M. KH_2PO_4 , pH 4.45. Substrate—0.01M. Glucose.

Temperature ° C.	4,6-dinitro-o-cresol 10 ⁻⁵ M.		2,4-dichlorophenol 10 ⁻⁴ M.		2,4,5-trichlorophenol 10 ⁻⁵ M.	
	Q _{O₂} control	Optimum percentage stimulation	Q _{O₂} control	Optimum percentage stimulation	Q _{O₂} control	Optimum percentage stimulation
15.0°	32	43			32	45
20.0°	47	58	48	62	46	54
25.0°	83	28	84	19	86	25
30.0°	99	18	98	6	97	12

Similarly, it is shown in Table II, that the percentage stimulation of oxygen consumption in yeast is larger at 15°C., or 20°C., than at 25°C., or 30°C.

TABLE III.
Stimulation of Oxygen Consumption of Rat Kidney Slices by Dinitroresol, Dichlorophenol, Trichlorophenol, and Triiodophenol. The Suspension Medium Was Ringer-phosphate, pH 7.35. Substrate—.02M Sodium Lactate.

Compound	Temperature ° C.	Q _{O₂} Control	Optimum molar concentration	Optimum percentage stimulation
4,6-dinitro-o-cresol	20.0°	7.3	10 ⁻⁵	68
	37.0°	26.0	10 ⁻⁵	43
2,4-dichlorophenol	20.0°	7.3	10 ⁻⁴	41
	37.0°	24.6	10 ⁻⁴	20
2,4,5-trichlorophenol	20.0°	7.4	10 ⁻⁵	34
	37.0°	23.3	None	None
2,4,6-triiodophenol	20.0°	7.4	10 ⁻⁵	14
	37.0°	18.4	None	None

The data of Table III show that each of 4 phenol derivatives gave a larger relative stimulation of lactate oxidation by rat kidney tissue slices at 20°C. than at the approximately physiological temperature of 37°C.

The experiments on yeast and rat tissues recorded here demon-

strate that the metabolic stimulating effects of dihalophenols and trihalophenols, which are relatively insignificant in the higher temperature ranges, are more readily recognized at lower temperatures. These experiments are also of interest in connection with the elucidation of the mechanism of oxidation stimulation. This subject will be more fully discussed at a later date.

8704 P

Further Observations on the Function of Supernumerary Fingers in Man.*

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In a previous communication by one of the authors,¹ the results of a study of finger movements in a reduplicated human hand, originally described by Halverson and Amatruda,² were reported. The supernumerary fingers 3, 4 and 5 exhibited a decided tendency to be moved each one in association with the corresponding normal finger. Recently, after an interval of 8 months, at the beginning of which the patient had been instructed as to how to attempt to acquire the independent use of homologous fingers, the case was reexamined.

Kymographic records by means of torsion wire levers were taken simultaneously from 4 fingers, the normal 3rd and 4th and the supernumerary 3rd and 4th, the lower arm being fixed in pronation or supination. In spite of the marked tendency of all fingers except the unpaired index to move *en masse*, it was noticeable that during random movements, as well as in attempts to counteract the passive extension or flexion of a given finger, homologous fingers would contract in unison, much in the way described in the former communication. It was equally clear, however, that *independent* movements of each finger of a homologous pair could also be obtained, provided the girl was brought to focus her attention on the task of dissociating. Then, normal 3rd and supernumerary 3rd

* Aided by the Biological Research Grant to the University of Chicago from the Rockefeller Foundation.

¹ Weiss, Paul, *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **33**, 426.

² Halverson, H. M., and Amatruda, C. S., *J. Gen. Psychol.*, 1935, **13**, 140.

Similar case described by Dwight, T., *Anat. Anz.*, 1893, **8**, 60.

could be moved alternately, as well as could normal 4th and supernumerary 4th. Visual control or touching of the finger to be moved independently greatly facilitated the dissociability. Fatigue, on the other hand, seemed to reduce it. The girl, according to her own statement, had always to spend a certain amount of effort on keeping homologous fingers from working in association.

On the whole, the conclusion seems to be warranted that cortical activity, as expressed by conscious effort and focussed attention, can momentarily abolish the association between homologous muscles existing on lower levels of central activity. It is obvious that the primary condition is the association while the dissociability is the result of secondary acute intervention of the cortex. Animals lacking a cerebral cortex such as the amphibia may therefore not be expected to show phenomena of secondary dissociation, and as a matter of fact have never thus far displayed any.³

8705 P

Mechanism of Increased Capillary Permeability in Inflammation.*

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When various types of inflammatory exudates obtained from dogs or rabbits are introduced into the normal skin of either of these animals an immediate vasodilatation and increased capillary permeability follow. This is demonstrated by the diffuse accumulation of a dye (trypan blue) from the circulating blood into areas treated in this way.^{1, 2, 3} This finding suggests that an active substance increasing capillary permeability is present in exudates. The effective agent may be recovered by treating the exudates with saturated ammonium sulphate or 20% sodium sulphate. It is thermostable and is active at pH 7.4 to 6.5. Dialysis of the

³ Weiss, Paul, *Ergebn. d. Biol.*, 1928, **3**, 1; *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **33**, 241.

* Read before the American Society for Experimental Pathology, Washington, D. C., March 26, 1936. Aided by grants from the Permanent Charity Fund, and the Wellington Research Memorial Fund, Harvard Medical School.

¹ Menkin, V., *J. Exp. Med.*, 1929, **50**, 171.

² Menkin, V., and Menkin, M. F., *J. Exp. Med.*, 1930, **51**, 285.

³ Menkin, V., *Am. J. Med. Sci.*, 1935, **190**, 583.

ammonium sulphate treated exudate yields a water soluble crystalline material in the evaporated dialysate which is fully potent, whereas the dialyzed material remaining in the cellophane bag is inactive. Direct dialysis of the untreated cell-free exudate likewise yields some of the active material in the evaporated dialysate. By this latter procedure, however, a small part of the active material remains in the cellophane bag apparently in a non-diffusible form. Since the active substance does not possess the properties of histamine, a definite objection is raised to the hypothesis of Sir Thomas Lewis which involves the action of histamine or an "H-like" substance.⁴ Histamine in the rabbit skin in varying concentrations dissolved in either water, saline, or serum causes central blanching and occasionally a peripheral accumulation of the dye from the blood stream in a "flare-like" formation. The active material found in exudates induces diffuse accumulation of dye throughout the treated skin area. Histamine contracts the guinea pig intestine. The active material from exudates, on the other hand, relaxes the isolated strip of intestine. These opposed effects would rule out the possibility that the active material in exudates is histamine or its closely related H-substance. Similar results as with the exudates of dogs and rabbits were obtained with a human exudate from a case of pleural effusion containing hemolytic streptococci. This exudate was obtained through the courtesy of Doctor H. L. Blumgart and Mrs. D. R. Gilligan of the Beth Israel Hospital, Boston.

The ashing of the exudate yields an aggregation of inorganic substances which when dissolved or suspended in water induce in the normal skin an almost immediate increase in capillary permeability. There is at present no available evidence that phosphates, iron, copper, aluminum, or silicon participate in this effect. The bulk of the ash consists of potassium, sodium, calcium, and magnesium. Potassium solutions, either as the chloride or the hydroxide, injected intracutaneously induce an accelerated passage of dye through the capillary wall in concentrations, however, that are higher than the amount of potassium recovered from an exudate. Chemical determinations show that the level of potassium is as a rule about twice as great in exudates than in blood serum. In this connection it may also be mentioned that either undiluted or ashed blood serum produces a weaker effect on the capillary wall than the corresponding inflammatory exudate. Potassium

⁴ Lewis, Th., *The Blood Vessels of the Human Skin and Their Responses*, Shaw and Son, Ltd., London, 1927.

salts, as in the case of exudates, depress the tonus of the guinea pig intestine. Magnesium, sodium, and calcium are found in no higher concentration in a non-purulent exudate than in the blood serum. Calcium, however, is found in higher concentrations in the later stage of inflammation when the exudate is frankly purulent. It is consequently merely suggestive that the greater effect obtained on the capillary wall by ashed exudate as compared with that elicited by similarly treated blood serum may in part be referable to the greater concentration of potassium in injured tissue as compared to that in serum. This increase in potassium concentration is found even in an exudate obtained during the first few hours of the inflammatory reaction. That other inorganic ions, besides potassium or perhaps in conjunction with it, may play a part either directly or indirectly in enhancing capillary permeability is as yet not precluded by the available data.

An attempt was also made to determine whether additional effective organic substances, other than histamine, are present in exudates. In view of the results with the previously described dialysis experiments, the end products of protein breakdown were studied. The level of amino acid concentration was usually found to be somewhat higher in exudates than in serum. This difference was invariably correlated by the more pronounced effect of the exudate in increasing capillary permeability. The total protein concentration in exudate and serum was shown to follow a trend opposite to that of amino acids. In other words there seems to be an inverse type of reciprocal relationship when one compares the concentration of total proteins and amino acids both in an inflammatory exudate and in the blood serum. This would suggest that proteolysis is very active in exudates yielding thus perhaps a number of intermediary products of protein catabolism. Proteose-peptone mixtures, peptone preparations, and certain of the alpha-amino acids have been found to increase capillary permeability when introduced into normal skin tissues.

The present observations indicate that in an inflammatory exudate certain of the inorganic ions, notably potassium found in increased concentrations, and various products of nitrogenous metabolism or intermediary protein catabolism, other than histamine, seem to play a rôle as substances connected with the mechanism of increased capillary permeability at the site of inflammation. These studies are being continued in an endeavor to purify and identify the active crystalline-like material isolated from an inflammatory exudate. The details of this investigation will be reported *in extenso* elsewhere.

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Alterations in Autarceologic Susceptibility to Experimental Poliomyelitis.*

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Dissemination of the virus of poliomyelitis is widespread, but the paralytic disease is limited; exposure tends to be uniform, while the occurrence of the frank disease is variable; infection tends to be general in the population, paralysis is selective.

The varying frequency with which paralytic poliomyelitis results from exposure to the virus indicates that autarceologic factors in the host largely determine the occurrence of paralysis. There are epidemiologic indications that autarcesis varies with climate and season, in different individuals in the same locality, and in the same individual. Other observations suggest that autarceologic susceptibility is dependent upon some inherent endocrine fault, which operates through its effect on a barrier to the virus (upper respiratory mucous membrane) or through some change in cellular affinities for the virus. That the former may be the case in the monkey and the latter in man is suggested by the fact that animals failing to develop the frank disease following repeated intranasal instillations of virus do not acquire subclinical immunity; whereas, there is epidemiologic evidence that this is the common occurrence in man.

Observations suggestive of the endocrine fault involved are the occurrence of the disease in certain types of individuals indicative of endocrine differences, as described by Draper; the familial occurrence of poliomyelitis¹; its occurrence following tonsillectomy²; and the frequency of the disease during pregnancy.³ Perhaps the single observation of a case in a castrate male is likewise suggestive in this connection.

In a discussion⁴ of the rôle of autarcesis in the epidemiology of poliomyelitis, it was suggested that some knowledge might be gained of the mechanism of this form of resistance by testing the influence

* This work was financed by the Harvard Infantile Paralysis Commission and the President's Birthday Ball Commission for Infantile Paralysis Research.

¹ Aycock, W. L., *West Va. Med. J.*, 1934, **30**, 11.

² Aycock, W. L., and Luther, E. H., *New Eng. J. Med.*, 1929, **200**, 164.

³ Aycock, W. L., *Trans. Sec. Ped., Am. Med. Assn.*, 1932.

⁴ Aycock, W. L., *J. Prev. Med.*, 1926, **3**, 245.

of appropriate endocrine alterations upon the outcome of intranasal instillations of virus in the monkey.

More recent developments in the field of endocrinology—thinning of the vaginal mucosa by castration and recornification following administration of estrin to castrate animals,⁵ the finding that the changes in the vaginal mucosa during pregnancy are of similar endocrine origin,⁶ together with evidence that the nasal mucosa participates in such changes⁷—are consistent with a number of epidemiologic and clinical indications concerning the mechanism of susceptibility to poliomyelitis, and suggest that castration would increase the susceptibility of monkeys to intranasal instillation of the virus, while the administration of estrin would, on the other hand, increase resistance.

Ten immature female monkeys were castrated. After 24 days, 5 of them were given daily for a period of 12 days an intramuscular injection of 2000 International units of estrin† in oil. Thirty-two days after castration, all 10 animals received in each nostril, daily for 6 days, 3 drops of a 5% suspension of poliomyelitis virus. Changes in the nasal mucosa have not yet been verified. The procedures, although designed to give maximal effects, are not known to be optimal.

Four of the 5 castrated animals not treated with estrin developed poliomyelitis; while only one of the 5 which had received subsequent injections of estrin succumbed. Three of 5 normal control monkeys developed the disease, a figure which is in keeping with our average experience with animals similarly inoculated. While in either case the numerical difference in the experimental as compared with the normal animals could hardly be considered significant, the fact that the experiment was double in nature—designed on the one hand to increase resistance, and on the other to increase susceptibility—affords the difference in the outcome in the 2 instances more significance than would be indicated by the actual numerical variation from the normal in either case.

⁵ Allen, Edgar, *Contr. to Embryo*, Carnegie Inst., Washington, 1927, **19**, 1.

⁶ Davis, M. E., and Hartman, C. G., *J. Am. Med. Assn.*, 1935, **104**, 279; Hisaw, F. L., *Anat. Rec.*, 1935, **64**, 54.

⁷ Pighini, G., and Porta, C. F., *Valsalva*, 1934, **10**, 140.

† Amniotin kindly supplied by E. R. Squibb and Sons.